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An Investigation Into The Relationship Between Dietary Acid Intake, Oral Hygiene Procedures And The Progression Of Erosive Tooth Wear

O'Toole, Saoirse

Awarding institution:
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AN INVESTIGATION INTO THE RELATIONSHIP BETWEEN DIETARY ACID INTAKE, ORAL HYGIENE PROCEDURES AND THE PROGRESSION OF EROSIVE TOOTH WEAR

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

SAOIRSE O'TOOLE

B DENT SC MFD RCSI

DEPARTMENT OF TISSUE ENGINEERING AND BIOPHOTONICS

KING'S COLLEGE LONDON DENTAL INSTITUTE

GUY'S HOSPITAL

LONDON

ABSTRACT

This thesis investigated conveniently selected factors that may influence the progression of erosive tooth wear. The designs were a laboratory study, case-control study and a RCT evaluating the influence of dietary advice on progression. The effect of timing of fluoride application with a previously reported erosive challenge was investigated in vitro. Human enamel samples (n=80) were treated with 225ppm stannous or sodium fluoride, either before or after a citric acid challenge (0.3%). The mean step heights (SD) for stannous fluoride applied before and after erosion were 3.2µm (0.57) and 4.2µm (0.7) respectively and these were statistically significantly lower than sodium fluoride application (before: 8.2µm (0.65) and after: 7.5µm (0.85), $p<0.001$). Stannous fluoride resulted in least step height when applied before erosion and sodium fluoride after erosion.

A validated questionnaire assessed commonly reported dietary and tooth brushing habits on a convenient sample of 300 participants with severe erosive tooth wear and 300 controls in a case-control study. The frequency of dietary acid intake between meals had the strongest association with erosive wear (OR 3.83-14.86, $p<0.001$). No association was observed with tooth brushing after an erosive challenge when dietary factors were controlled for.

A randomised controlled trial assessed the impact of enhanced dietary advice (n=28) on severe erosive tooth wear progression compared to standard of care advice (n=29). Addition-silicone impressions and questionnaires were taken at baseline and 6 months later. Impressions were cast in dental stone, scanned using

laser profilometry and superimposed using surface matching software. The dietary intervention group reduced daily frequency of acid intake between meals by three intakes (IQR 1, 3) compared to one intake (IQR 0, 3) for controls, $p=0.048$. The intervention group also demonstrated reduced volume loss per surface (0mm^3 (IQR -0.18, 0.18)) compared to controls (-0.06mm^3 (IQR -0.24, 0.11), $p=0.045$).

These studies suggest that prevention should focus on limiting dietary acid consumption between meals.

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PREFACE

This thesis is an investigation into the timing of dietary acid intake, oral hygiene procedures and tooth wear. The aim was to increase the evidence base for preventing dietary erosive tooth wear progression using a combination of laboratory, epidemiological studies and interventional studies.

The literature review in Chapter one overviews the current evidence base for risk factors associated with dietary erosive tooth wear. Current concepts regarding surface protection, abrasion and remineralisation are discussed. Methods of dietary assessment, dietary advice and adherence to advice were also reviewed. The measurement of erosive tooth wear both based in the laboratory and clinically, are challenging and methods were critically reviewed.

An in vitro investigation into the optimal timing of fluoride application, either before or after an erosive challenge, is described in Chapter two. This chapter also highlights how changing the research methodology can produce different research outcomes.

Chapter three describes the training and standardisation exercises for the Basic Erosive Wear Examination (BEWE), in addition to the development and validation of a dietary assessment questionnaire. The BEWE and dietary questionnaire underpinned the clinical studies described in chapters four and five.

The questionnaire utilised a case-control design to investigate the association between timing of dietary acid intake, oral hygiene procedures and erosive tooth wear. The methodology, findings and discussion is described in Chapter four.

The risk factors identified from chapter four were used to design a randomised controlled clinical trial investigating the impact of dietary advice on tooth wear progression. An inter-disciplinary approach was used to develop a behaviour change intervention which was compared to standard-of-care dietary advice. This assessed if providing advice can result in behaviour change and whether this behaviour change is sufficient to slow tooth wear progression. Furthermore, a novel method of measuring tooth wear in vivo was utilised. The development of the intervention, methodology and problems identified with measurement of erosive wear in vivo are described in Chapter five.

Chapter six provides a general discussion and reflection on the findings while Chapter seven discusses suggestions for future work.

CHAPTER 1: LITERATURE REVIEW

1.1 TOOTH WEAR

Tooth wear is a complex process involving erosion, attrition, abrasion and theoretically, abfraction. Erosive tooth wear is a relatively new term used to describe the interaction of acids with mechanical tooth wear. It has recently been defined as the chemical-mechanical process resulting in a cumulative loss of hard dental tissue not caused by bacteria (Carvalho *et al.* 2015). Epidemiological evidence suggests that the prevalence of erosive tooth wear is increasing, particularly in younger age groups (Jaeggi and Lussi 2014).

1.1.1 *EROSION*

Erosion is defined as the progressive loss of tooth substance by chemical processes that do not involve bacterial action (The Academy of Prosthodontics 2005).

Erosion presents as a smooth, silky or glazed surface with slight changes to the original morphology such as rounding of cusp tips and loss of developmental ridges. As erosion progresses distinct defects in the enamel may develop. Often the width of the lesion extends beyond the depth. As the enamel thins, crown height may be reduced and incisors may be prone to incisal chipping. Increased erosion wear rates on teeth may leave restorations raised above the level of the dental surface. The clinical presentation of erosion may vary depending on the aetiology and site affected (Lussi 1996; Nunn *et al.* 2003; Wetselaar and Lobbezoo 2016).

In addition to clinical findings, dental hypersensitivity is increasingly being recognised as a symptom of active erosion (Bartlett 2016a). A recent *in vivo* study observed an increase in clinical dentine hypersensitivity when a dietary acid was

consumed in the previous hour (Olley *et al.* 2015). Dietary acids have the potential to remove the smear layer, opening dentinal tubules and increasing the risk of hypersensitivity (West *et al.* 2013a). Although large epidemiological studies have linked the presence of dental hypersensitivity with an acidic diet (West *et al.* 2013b) there are clinical studies which observed no relationship (Mafla and Lopez-Moncayo 2016). Further clinical studies would be beneficial to confirm the role of erosion in dental hypersensitivity.

1.1.1.1 Extrinsic causes of dental erosion

The most common extrinsic cause of dental erosion and the focus of this thesis are dietary acids. The chemical and behavioural factors which affect the erosive potential of a dietary acid are discussed in this section. There are other rare extrinsic causes of dental erosion such as acid fumes in factories (Petersen and Gormsen 1991; Chaturvedi 2015) and inadequately maintained chlorinated swimming pools (Zero 1996; Buczkowska-Radlińska *et al.* 2013) which are outside the scope of this thesis.

1.1.1.1.1 Specific dietary acids

The chemical erosive potential of an individual dietary acid depends on the pH value, titratable acidity, calcium chelation properties, buffering capacity and mineral content (Barbour *et al.* 2011). The pH value is the most widely used predictor of erosive potential, particularly when assessing the inchoate erosive challenge (Shellis *et al.* 2014). Authors have argued that titratable acidity better characterises the erosive potential during longer exposure times (Hannig *et al.* 2005; Jensdottir *et al.* 2006) but both provide information about the erosive potential of an acid. Citric acid, the most common form of dietary acid, has been particularly implicated in dietary erosive tooth wear (Shellis *et al.* 2013). This

complex, weak acid partly dissociates depending on the ion saturation of the environment. If protons are consumed during the erosive challenge, further protons can be released. Furthermore, citric acid chelates with calcium, forming complexes of calcium citrate with varying degrees of solubility (Shellis *et al.* 2014). To chelate with calcium, citric acid molecules must have delivered at least two of its three protons. Citric acid releases protons at pH 3.13, 4.74 and 6.42. This results in the majority of calcium chelation occurring as the pH rises above pH 4 (Shellis *et al.* 2013). At this pH, up to 32% of the calcium in saliva can be complexed to citrate rendering it inactive for remineralisation (Meurman and ten Cate 1996).

The majority of in vitro studies contrast the erosive potential of different acidic foods and beverages based upon the above parameters (Wang and Lussi 2012). Citrus fruits, other fruits, fruit juices, fruit-flavoured waters, fruit-flavoured tea, most carbonated beverages including sugar-free versions, energy drinks, sports drinks, acidic sweets, vitamin C supplements and most alcoholic beverages have been implicated (Järvinen *et al.* 1991; Ireland *et al.* 1995; Ganss *et al.* 1999; Lussi *et al.* 2000; Rios *et al.* 2009; Wang and Lussi 2012). Acidic medications and their artificially sweetened counterparts have also been implicated, particularly when consumed on a daily basis (Attin *et al.* 2001; Adrian Lussi, Megert, *et al.* 2012).

Clinical studies have attempted to investigate if a specific dietary acid has a stronger relationship with erosive tooth wear. A trans-European study on 3,187 adults observed a highly significant ($p < 0.0001$) relationship between frequent fresh fruit intake and presence of tooth wear (Bartlett *et al.* 2013). A study investigating 1,456 subjects in Norway reported an increased odds ratio for drinking sugary soft drinks (OR=1.9) over fruit juices (OR=1.6) (Mulic *et al.* 2012).

Correr *et al.* 2009 observed an odds ratio of 1.12 for artificial juice consumption compared to 2.09 for soft drink consumption in a cross-sectional study on 389 children (Correr *et al.* 2009). Another UK study on 2,385 children observed that consumption of pickles had the strongest association with increased tooth wear (Milosevic *et al.* 2004).

These clinical trials are highly dependent on the diet of the population being studied and the questions asked by the examiner. Studies have suggested that a preference for acidic foods alone is related to erosive tooth wear (O'Sullivan and Curzon 2000; Dugmore and Rock 2004a). While the erosive potential of specific dietary acids is important, acids are regularly consumed by the population without resulting in pathological wear. Other factors may play a more important role in the progression of erosive tooth wear.

1.1.1.1.2 Frequency of dietary acid intake

There is evidence, from laboratory and epidemiology studies, to suggest that frequency of dietary acid consumption may be the most important risk factor in the development of dietary erosive tooth wear. Laboratory studies have shown that increased frequency of erosive cycles result in greater bulk tissue loss (Mistry *et al.* 2015). Several epidemiological studies have observed increasing odds ratios with increasing frequency of dietary acid intake (Bardsley *et al.* 2004; Dugmore and Rock 2004a; Milosevic *et al.* 2004; El Aidi *et al.* 2011; Mulic *et al.* 2012; Lussi and Hellwig 2014).

One case-control study (n=200 adults) reported participants were 19 times more likely to have erosive wear if citrus fruits were consumed more than twice daily and 14.2 if soft drinks were consumed twice daily (Järvinen *et al.* 1991). However,

the sample size was relatively small and confidence intervals quite large to extrapolate these large odds ratios to the general population. Mulic *et al.* 2012 used a cross-sectional study design on 1,456 18 year olds and observed the odds ratio with erosive tooth wear increased from 1.5 for one daily intake of sugary soft drinks to 2.2 if consumed several times daily (Mulic *et al.* 2012). Other studies have observed a relationship between erosive tooth wear and two or greater daily intakes of acidic beverages (Moazzzez *et al.* 2000; Milosevic *et al.* 2004) or three or more daily intakes of acidic beverages (Dugmore and Rock 2004a; Murakami *et al.* 2011; Abu-Ghazaleh *et al.* 2013). These studies are limited in their ability to assess risk for a combination of different sources of dietary acid intake e.g. combinations of beverages and acidic fruits. There are epidemiological studies observing no relationship between frequency of dietary acid intake and erosive tooth wear (Chadwick *et al.* 2005; Tahmassebi *et al.* 2006; Alvarez Loureiro *et al.* 2015). However, when assessing their methodology, the frequency of acid intake is questioned on a weekly basis rather than a daily basis (Ayers *et al.* 2002; Ratnayake and Ekanayake 2010; Mafla and Lopez-Moncayo 2016). Risk of erosive tooth wear appears to increase significantly with daily consumption (Sovik *et al.* 2015; González-Aragón Pineda *et al.* 2016) although this remains to be verified. In summary, there is considerable evidence to substantiate the belief that the frequency of dietary acid consumption is associated with erosive tooth wear.

1.1.1.1.3 Quantity of dietary acid intake

The majority of epidemiological studies have focused on the frequency of acid intake but with limited assessment of quantity of acid intake. Quantity of dietary acid intake is relatively difficult to assess as portion size is often subjective and difficult to measure (Andersen *et al.* 2004). Perhaps the most sophisticated method

to date has been performed by Sovik *et al.* when the quantity was assessed via a self-administered questionnaire after participants were asked to report the quantity of each drink in litres. Acidic beverage consumption was categorised into low (0-0.24 L/day) moderate (0.25-0.74 L/day) and high (0.75-5 L/day) consumption (Sovik *et al.* 2015). A higher prevalence of erosion was observed in those with increased quantity consumption. Another cross-sectional study performed on young Icelandic adults dichotomised quantity data into > 1 L and < 1 daily. A relationship with erosive tooth wear was observed when greater than 1 litre of carbonated drinks were consumed (Jensdottir *et al.* 2004), although limited information is given on the method of data collection in this study. Studies have also measured quantity in litres consumed per year (Johansson *et al.* 2002; Hasselkvist *et al.* 2014). The interpretation of this as a meaningful guideline to patients is difficult and gives no indication as to frequency. El Aidi *et al.* assessed beverage intake in adolescents via glasses per week and observed that the number of glasses of carbonated beverages consumed was statistically associated with erosive wear (El Aidi *et al.* 2011). This method may not be reliable, having been obtained from a self-administered questionnaire, where clear instructions were not given about glass size. The only study, to the author's knowledge, investigating quantity of fruit intake and erosive wear was performed in an investigation of prevalence of erosion in those consuming a raw food diet (Ganss *et al.* 1999). The quantity of fruit intake, via a self-administered questionnaire, was assessed with picture accompaniments providing guidance as to portion size. An increased prevalence in those with a median fruit intake of 9.5 kg (Range 1.5-23.7 kg) per week was observed (Ganss *et al.* 1999). There are clinical studies which have found no relationship between quantity of acid intake and erosive tooth wear (Mathew *et*

al. 2002) and the field would benefit with input from a dietician or other expert in dietary assessment, to optimise assessment of quantity of acid intake.

1.1.1.1.4 Timing of Dietary Acid Intake

It has been recommended to consume dietary acids at mealtimes to minimise potential damage to dental tissues (Lussi, Jaeggi, and Zero 2004), although there is a lack of clinical data to support this. Theoretically, increased salivary flow rates and buffering capacity of additional foods at mealtimes may lower the erosive potential of the acid sufficiently to prevent demineralisation and irreversible tissue loss (Moynihan and Petersen 2007). Erosive wear may also be decreased when dietary acids are consumed with foods containing a high calcium or phosphate content (Lussi, Jaeggi, and Zero 2004). Some epidemiological studies have observed a protective effect with high consumption of dairy produce (El Aidi *et al.* 2011; Salas *et al.* 2015; Hasselkvist *et al.* 2016) and others have not (Bartlett *et al.* 2013; Okunseri *et al.* 2015). To the author's knowledge there has been one study investigating the effect of consumption of acidic beverages between meals (Hasselkvist *et al.* 2016). The frequency of carbonated beverage consumption between meals was observed to be associated with erosive tooth wear progression ($p=0.018$). Disappointingly, reporting on this individual aspect is not clear as it was a large prospective longitudinal study investigating several other variables. The protective effect of consuming acids with meals remains to be investigated epidemiologically and the clinical significance remains to be verified.

1.1.1.1.5 *Duration of dietary acid intake and alternative drinking methods prior to swallowing*

Alternative habits prior to swallowing, such as holding drinks in the mouth, swishing or rinsing drinks, or sipping drinks slowly have also been associated with erosive wear. One of the first studies to investigate alternative drinking habits examined participants' dental plaque pH. The author's observed that intraoral plaque pH decreased to a greater extent when a carbonated beverage was rinsed in the mouth compared to "normal" drinking (Edgar *et al.* 1975). A more recent study investigated different forms of drinking: long-sipping, short sipping, holding and gulping and observed larger drops in pH when drinks were held in the mouth, but a sustained lower pH when long-sipping was performed (Johansson *et al.* 2004).

Following an acid challenge, the liquid surface layer adjacent to the tooth becomes saturated with calcium and phosphate ions removed from the dental surface (Lussi *et al.* 2011). Provided this layer remains undisturbed an equilibrium can be established when the demineralisation process of tooth structure stops (Shellis *et al.* 2014). Following cessation of the acid intake, acid clearance and normalisation of the intraoral pH has been reported to occur rapidly over 2-13 minutes (Millward *et al.* 1997; Bartlett *et al.* 2003; Hans *et al.* 2016). However, this saturated surface layer may be disrupted in vivo as the acid is replenished through prolonged intake or if the acid is forcefully moved around the mouth with "swishing" or "rinsing" habits. This theory is supported in vitro. Shellis *et al.* observed increased tooth wear in vitro when the duration of acidic challenge was increased, the acid was concentrated in one area, or the acid was agitated against dental surfaces (Shellis *et al.* 2005). An increased flow rate and the adherence of the acid to the surface has also been reported to result in increased wear in vitro (Ireland *et al.* 1995;

Busscher *et al.* 2000). This may be particularly relevant for carbonated beverages, where the increased activity of the effervescent acid may help to drive the erosive process (Busscher *et al.* 2000).

Clinically, prolonged drinking time of an acidic beverage has been observed to result in increased wear. Hara *et al.* reported that enamel surfaces with a salivary pellicle were able to reduce demineralisation by orange juice for up to 10 minutes of acid exposure compared to surfaces with no pellicle. In contrast, the protective effect of the pellicle failed after a 20 minute acid exposure (Hara *et al.* 2006).

Johannsson *et al.* 2002 observed that those with erosion held the acidic drink in their mouths for statistically longer prior to swallowing (Johannsson *et al.* 2002), although this was a small sample size of 20 adults. A larger study of 354 adolescents reported an increased risk of erosive tooth wear when participants “made the drink last” compared to those who “drank straight away” (Al-Majed *et al.* 2002). In contrast one study reported that participants with erosive wear drank more quickly than a control group (Moazzez *et al.* 2000). The authors also reported that the intraoral pH remained lower for longer on the lower first molars on participants with erosive wear. The authors hypothesised that this may be indicative of a habit of retaining the drinks in the mouth as the group observed no difference between salivary parameters.

Studies which have investigated this epidemiologically have observed statistical relationships between alternative drinking habits and tooth wear (O’Sullivan and Curzon 2000; Bartlett, Fares, *et al.* 2011; Chrysanthakopoulos 2012; Hasselkvist *et al.* 2016). The relationship between prolonged fruit eating habits and erosive tooth wear has yet to be investigated epidemiologically.

1.1.1.2 Intrinsic causes of dental erosion

The pH of gastric acid normally ranges from 1-3 with hydrochloric acid being the predominant acid (Lindquist *et al.* 2011). Release of the gastric contents into the oral cavity due to an underlying physical or mental condition has been associated with erosive tooth wear (Moazzez and Bartlett 2014). As detailed discussion of the intrinsic causes of erosive tooth wear is outside the scope of this thesis, this review will focus on potential difficulties when attempting to exclude intrinsic aetiological factors.

Conditions implicated in erosive tooth wear may be recognised from taking a thorough medical history (Barbosa *et al.* 2010; Moazzez and Bartlett 2014).

Difficulty arises when the disease is undiagnosed or the patient does not divulge their condition. The most common intrinsic cause of erosive wear is gastro-oesophageal reflux (Moazzez and Bartlett 2014). Symptoms which provide an indicator of gastro-oesophageal reflux include heartburn, chest pain, chronic cough, hoarseness and globus (Moazzez and Bartlett 2014), although it is known that symptoms are not necessarily a reflection of the severity of the disease (Quitadamo *et al.* 2015). Symptoms may also not be present (Bartlett *et al.* 1996). If symptoms are present, the general population do not always seek medical attention for it (Cohen *et al.* 2014) and as a result the disease may remain undiagnosed and uncontrolled (Cohen *et al.* 2014). Investigative tests to confirm a diagnosis of reflux are invasive and it is therefore difficult to diagnose gastro-oesophageal reflux as the aetiological factor in erosive tooth wear if the patient is unaware of it (Bartlett *et al.* 1996; Bartlett *et al.* 2001). This is particularly true if patients are also consuming an acidic diet.

Eating disorders are also common in the UK population with bulimia nervosa being of particular significance to dental erosion. Bulimia nervosa is reported to affect 0.5% of women and 1% of men in the UK (Wiles *et al.* 2006). A recent systematic review observed those with eating disorders to be 5 times more likely to have dental erosion (95% CI 3.31-7.58)(Kisely *et al.* 2015). Co-morbidities often present alongside eating disorders which can exacerbate erosive tooth wear such as depression, anxiety and poor diet (Kisely *et al.* 2015). Anti-depressant medication has been implicated in erosive tooth wear possibly due to xerostomic side-effects of the medication (Bartlett *et al.* 2013). Klein *et al.* also observed in a case-control study that bulimia nervosa patients (n=78) consumed on average between 25.4 – 39.5 cans of diet beverages per week compared to 7.4 cans for healthy controls (n=32) (Klein *et al.* 2006). In addition, bulimic patients have also been observed to have lower unstimulated salivary flow rates (Dynesen *et al.* 2008; Uhlen *et al.* 2014; Kisely *et al.* 2015). Similar to gastro oesophageal reflux disease, studies have reported that severity and duration of disease is not always an indicator of severity of wear (Schlueter and Tveit 2014; Uhlen *et al.* 2014). Patients may also be reluctant to divulge their eating disorder to their dentist, again creating difficulties with differentiating intrinsic erosion from extrinsic erosion (Burkhart *et al.* 2005). These potential confounding factors need to be taken into consideration when investigating dietary erosive wear.

1.1.2 ABRASION

Abrasion describes the mechanical removal of dental hard tissues through the use of foreign objects or substances (Imfeld 1996a). Abrasion lesions have been quoted as manifesting as wedge shaped defects on the buccal cervical surface of canines and premolars. Lesions are often more wide than deep (Ganss and Lussi

2014). Tooth brushing with an abrasive dentifrice, nail biting, pen biting, toothpicks and other foreign objects have been implicated in abrasive wear (Addy and Hunter 2003). Of these tooth brushing is the most common with the strongest evidence base (Wiegand and Schlueter 2014). Although tooth brushing with a dentifrice of high abrasivity alone can result in tooth wear (Dzakovich and Oslak 2008), there is often an erosive element involved (Addy 2005) and this is discussed further in section 1.3.3.2.

1.1.3 *ATTRITION*

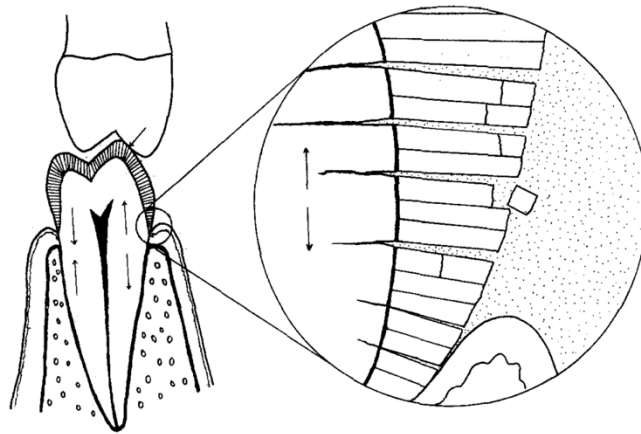
Attritional wear is the loss of tooth tissue due to friction between opposing teeth (Van'T Spijker *et al.* 2007). Attritional wear can be physiological due to normal wear and tear or pathological (bruxism) and is characterised by flattened occlusal surfaces with interdigitating wear facets on the opposing arch. The degree of wear in both arches are generally equal as is the degree of wear in enamel and dentine (Bartlett 2005). There may also be fracturing of cusps or restorations. Intraoral soft tissue signs can include white keratinization lines on the buccal occlusal line and crenations on the tongue (Bartlett 2005; Wetselaar and Lobbezoo 2016). Extra oral signs may include masseteric hypertrophy, tenderness of the muscles of mastication and limited opening. The patient may also report with temporomandibular dysfunction symptoms such as morning stiffness/pain and headaches (Jonsgar *et al.* 2015).

1.1.4 *ABFRACTION*

Abfraction is defined as wear at the cemento-enamel junction occurring when enamel prisms fracture due to concentration of stress on the cervical region during function and parafunction (Lee and Eakle 1984). Lee & Eakle 1984 described the process as weakening of the cemento-enamel junction due to eccentric occlusal

forces which made enamel more susceptible to erosive and abrasive forces (Figure 1, Lee and Eakle 1984).

Figure 1: Theoretical aetiology of abfraction



Grippio was the first to term the process “abfraction” in 1991 and classified it as a purely mechanical process from premature occlusal contacts (Grippio 1991).

Unfortunately, this has resulted in some practitioners forming unnecessary occlusal equilibrations in an attempt to prevent tooth wear progression (Wood *et al.* 2008).

To date there is little evidence to support abfraction. One study performed extensive investigations on the anatomy of cervical lesions and could not find evidence to support a microfracture theory (Walter *et al.* 2014). Experimental evidence has shown that cervical tooth structure is more susceptible to degradation than occlusal enamel (Dejak *et al.* 2003) and is a confounding factor which is rarely accounted for in the limited number of clinical trials investigating abfraction. Furthermore, a recent systematic review concluded that there was limited evidence to support occlusal interferences as an aetiological factor in tooth

wear (Silva *et al.* 2013). Further research is required to prove that occlusal interferences are an aetiological factor in tooth wear progression.

1.1.5 *EROSIVE TOOTH WEAR*

Although the three wear processes are discussed separately above, clinically they rarely occur in isolation (Bartlett 2005). It has been recognised that early presentation with severe tooth wear will have some form of underlying erosive element (Margaritis and Nunn 2014). Erosive tooth wear is recognised as pathological wear facilitated by erosion (Carvalho *et al.* 2015). Erosive tooth wear may present with a combination of the histological features and symptoms from erosion, attrition and abrasion, which aid in the diagnosis of the primary aetiological factor.

The symptoms and complaints of erosive tooth wear vary between individuals (Al-Omiri *et al.* 2006). In some cases where severe wear may be present, the patient may remain asymptomatic and unconcerned. In other cases the wear may be quite minimal but is of concern to the patient (Al-Omiri *et al.* 2006). The clinical problems associated with tooth wear tend to be appearance, loss of function and pain (Al-Omiri *et al.* 2006; Daly *et al.* 2011; Wazani *et al.* 2012; Ahmed *et al.* 2014). A study on 290 tooth wear participants referred into Liverpool Dental Hospital restorative clinics reported aesthetics to be the most prevalent presenting complaint (59%), followed by hypersensitivity (40%), functional problems (16.6%), tooth or restoration failure (16.6%) and pain (13.8%). A similar recent study in Glasgow reported that 25% of patients reported hypersensitivity and 12% of patients reported impaired function. Few patients (8%) required treatment by a prosthodontic specialist (Ahmed *et al.* 2014). However studies have reported that

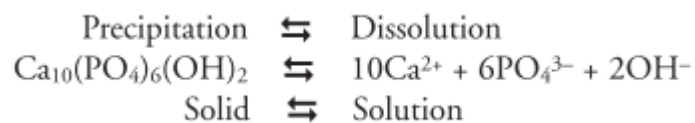
severe tooth wear can have a negative impact on quality of life comparable to the impact of being edentulous (Papagianni *et al.* 2013; Li and Bernabé 2016). Unlike caries, there is no clear indication when treatment is suitable and no clear consensus amongst experts in the field (Van'T Spijker *et al.* 2007; Bartlett and Dugmore 2008; Ganss 2008). When restorative treatment for severe erosive wear is required, it is frequently complex (Muts *et al.* 2014) and in severe cases often a full mouth rehabilitation approach is required. There is a direct conflict between the natural reluctance to remove further tooth structure with extensive preparations and the necessity for long-lasting restorations in an aggressive oral environment (Bartlett 2016b). There is also a paucity of critical reviews and high quality literature related to the long-term outcome of tooth wear rehabilitation approaches. If the patient is asymptomatic with no aesthetic concerns and function is not impaired, prevention of future progression may be the optimal treatment (Bartlett 2016b).

1.2 TIMING OF ORAL HYGIENE PROCEDURES

1.2.1 THE CHEMISTRY OF DENTAL EROSION

Enamel is a highly mineralised crystalline structure of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) and several impurities such as sodium, magnesium, and fluoride which vary between individuals (Lussi *et al.* 2011). Hydroxyapatite (HA) is insoluble in water but is susceptible to dissolution in acidic conditions. The critical pH of HA is the pH at which the surrounding solution is just saturated with minerals with respect to HA and depends on calcium, phosphate and other active ion concentrations in the solution (Shellis *et al.* 2014). When the pH of a solution drops below this level of saturation, HA will dissolve until the solution reaches saturation again. Once a critical pH has been reached initial dissolution occurs as shown Figure 2 (Dawes

Figure 2: Dissolution reaction of hydroxyapatite



2003).

When there is a supersaturation of calcium and phosphate ions the pH can be as low as 4 without damaging dental enamel. A commonly given example is that despite having a low pH due to the lactic acid content, yoghurt has low erosive potential (Lussi, Jaeggi, and Zero 2004).

Liquid can move through enamel prisms of the teeth causing deeper layers of softening (Bertacci *et al.* 2007). Mineral release from HA results in surface softening of the outermost layer between 0.2 and 2µm thick (Barbour *et al.* 2005; Lussi *et al.* 2011). At this stage, the tissue is particularly susceptible to mechanical forces (Lussi *et al.* 2011). In the absence of further erosive challenges or

mechanical removal an adaptive process can occur whereby minerals can form new ionic bonds within the crystalline structure (Lussi, Hellwig, *et al.* 2012).

1.2.2 AN OVERVIEW OF THE ROLE OF SALIVA IN EROSIVE TOOTH WEAR

There are several inherent protective mechanisms that limit hydroxyapatite dissolution during an erosive challenge. Saliva is thought to be the most significant protective factor with erosive challenges in situ or in vivo producing substantially less erosive wear than erosion in vitro (Hunter *et al.* 2000).

1.2.2.1 Preventing demineralisation

The presence of saliva initially acts on a macro scale within the oral environment, limiting the severity of the acid challenge. Presence of saliva dilutes the acid and gradually eliminates the acid from the oral cavity through swallowing (Buzalaf *et al.* 2012). Intraoral acid clearance rates in those with normal salivary flow rates have been observed to range from 2-13 minutes (Millward *et al.* 1997; Bartlett *et al.* 2003; Hans *et al.* 2016). Higher salivary flow rates have been associated with lower plaque pH after an erosive challenge (Tenovuo and Rekola 1977). In addition, studies have reported those with reduced salivary flow rate showed increased susceptibility to erosive wear (Dugmore and Rock 2003a; Moazzez *et al.* 2004; Dynesen *et al.* 2008). In contrast, some studies have observed increased erosive wear in those with a low salivary buffering capacity and not salivary flow rate (Moazzez, Smith, and Bartlett 2000; Lussi *et al.* 2012). As saliva is supersaturated in calcium, phosphate and other minerals with respect to tooth structure, it is able to act as a buffer, neutralising acids (Lussi *et al.* 2011).

Saliva also acts on a micro scale on the dental surface. The acquired salivary pellicle is the protein-based layer, which rapidly forms on teeth immediately after contact with saliva. It forms through the selective adsorption of approximately 130

salivary proteins to enamel surfaces (Siqueira *et al.* 2012). The role of individual proteins present in the pellicle is increasingly being researched and some proteins e.g. statherin, mucin, may be more of relevance than others in protecting against acid damage (Moazzez *et al.* 2014). This protein layer initially acts as a diffusion membrane preventing direct contact of the acid on the hydroxyapatite crystals (Carpenter *et al.* 2014) adding protection even at the initial stages of maturation (Hannig *et al.* 2004; Hannig *et al.* 2009). It reaches an initial thickness after 2-3 minutes and stays at that level for a period of approximately 30 minutes. It then triples its thickness and stabilizes at this size (Skjørland *et al.* 1995). This thickness is reported to be between 0.3µm and 1.06µm (Amaechi *et al.* 1999) and may be an important indicator in the susceptibility of sites to dental erosion (Amaechi *et al.* 1999). The pellicle has not been reported to be removed by normal tooth brushing force and dentifrices with medium/low abrasivity (Joiner *et al.* 2008).

It is clear that salivary factors have a role in protection against erosive tooth wear and it is likely to be a combination of mineral and protein content, stimulated and unstimulated flow rates and buffering capacity (Buzalaf *et al.* 2012). However, high individual variation is common in salivary studies and different donors provide different levels of protection despite having similar salivary parameters (Wetton *et al.* 2007; Lussi *et al.* 2014). This may be an explanatory factor for erosive wear progression in some individuals and not others.

1.2.2.2 Encouraging remineralisation

Both artificial and natural saliva have been observed to increase the surface hardness of enamel and dentine following an erosive challenge (Attin *et al.* 2000; Amaechi and Higham 2001; Attin *et al.* 2001). However, dental impressions taken immediately after and at increasing time intervals post an erosive challenge

observed no statistical visual differences in SEM imaging after a one hour in situ period (Rios *et al.* 2008). Another clinical SEM study observed remineralisation had started to occur within 2 hours and showed significant signs of repair at 24 hours (Seong *et al.* 2015). However, full rehardening of enamel/dentine with saliva under realistic clinical conditions has not yet been reported. One clinical study performed acid etching on premolar teeth scheduled for extraction for 2 minutes with 50% phosphoric acid. When extracted 90 days later and subjected to SEM imaging, evidence of etching was still present (Garberoglio and Cozzani 1979). Although this study had a small sample size (n=6) with an extreme acid challenge, it provides evidence that the remineralising potential of saliva is limited. More recent evidence of this can be observed in a quantitative in situ clinical experiment, where control enamel samples were eroded and left undisturbed in situ for 7 days without regaining their original microhardness value (Joiner *et al.* 2014).

Furthermore there are studies which suggest that proteins within saliva may interfere with the remineralisation process (Lussi *et al.* 1988). A recent paper observed that a 4 hour intraoral remineralisation period did not significantly increase abrasion resistance whereas a similar remineralisation period with artificial saliva did increase abrasion resistance (Lussi *et al.* 2014). The authors theorised that proteins can bind to the demineralised enamel acting as a potential barrier to remineralisation (Lussi *et al.* 2014). Further research is required in this area.

1.2.3 THE ROLE OF ORAL HYGIENE IN EROSIVE TOOTH WEAR

1.2.3.1 Fluoride therapy

There is a strong evidence base for using fluoride to prevent demineralisation and encourage remineralisation in the dental caries process. However the relationship between fluoride and the erosive process has yet to be fully clarified. Although both processes involve dental mineral loss, with interchanging degrees of demineralisation and remineralisation, the caries lesion is different to the erosive lesion. Caries most commonly occurs in areas of plaque stagnation, presenting in the sheltered areas of fissures, interproximally and at gingival margins. Formation of fluoride reservoirs surrounding an affected area is possible and oral hygiene is commonly poor. In contrast, erosive lesions are found on exposed surfaces, most commonly affecting the smooth surfaces of upper anterior teeth and the occlusal surfaces of first molars (Jaeggi and Lussi 2014). Erosive lesions can be diffuse, widespread lesions affecting all teeth in the dentition to a greater or lesser degree. Poor oral hygiene is not necessarily implicated; dental erosion and good oral hygiene performed after an erosive challenge may be related to increased dental tissue loss (Lussi and Carvalho 2014). There are limited evidence based guidelines when targeting erosive wear surrounding oral hygiene procedures and there is no consensus on evidence based guidelines for fluoride therapy.

1.2.3.1.1 Mechanisms of action of fluoride

Despite this, there is evidence that fluoride has a preventive role in erosive tooth wear (White *et al.* 2012). The potential mechanisms of action of fluoride against erosive wear are threefold. Incorporation of fluorides into the crystal lattice of enamel hydroxyapatite has been shown to reduce susceptibility to future erosive challenges (Schlueter, Klimek, *et al.* 2009a; Lussi and Carvalho 2015). Secondly, the formation of calcium fluoride deposits on the surface providing sacrificial intraoral

fluoride ions, may act as a potential barrier against an acid challenge (Gerth *et al.* 2007; Lussi, Hellwig, *et al.* 2012). Thirdly, following demineralisation, fluoride minerals may re-enter the crystalline structure, remineralising the eroded tissue (Barlow 2009).

However, the clinical relevance of these mechanisms in isolation is relatively unknown. Mineral incorporation has been observed to have a relatively weak protective effect compared to the presence of available fluoride ions (Ogaard *et al.* 1988). The presence of calcium fluoride deposits may also be limited. Under optimum conditions, Koeser *et al.* 2014, reported that coverage of no more than 40% of enamel surface can be achieved (Koeser *et al.* 2014). Furthermore retention of these precipitates is unlikely during repeated or severe erosive challenges possibly due to dilution or mechanical wear (Ganss *et al.* 2007; Austin *et al.* 2011; Austin *et al.* 2014). In addition, it has been recognised that once demineralisation has occurred it is very difficult to remineralise completely (Lussi *et al.* 2014). Theoretically, provided loss of the hydroxyapatite crystal scaffold has not occurred, ionic bonds may reform with minerals and ions present in the immediate environment. Fluoride application following an erosive challenge has been found to result in rehardening, as tested through hardness measurements (Huysmans *et al.* 2014).

There are those who believe that fluoride does not have a protective role in erosive tooth wear (ten Cate *et al.* 1998; Larsen and Richards 2002). A study by Larsen and Richards with the title “Fluoride is unable to reduce dental erosion from soft drinks” is frequently cited to augment the claim that fluoride cannot play a protective role in erosive tooth wear (Larsen and Richards 2001). For this study,

calcium fluoride salts were dissolved in erosive beverages and then two whole teeth were agitated in the acidic solution for 48 hours. Microradiography was then used to compare the erosive lesion depth against control beverages (n=2) without the calcium fluoride precipitates. It is unsurprising, given the low sample size and non-clinically relevant study design that no protective effect was observed.

There is evidence that the benefits of fluoride may be dose responsive (White *et al.* 2012). Additional use of fluoride mouthrinses in conjunction with the use of fluoride toothpastes has been reported to increase both fluoride availability and the level of fluoroapatite formation (Van Strijp *et al.* 1999). Increased levels of protection have also been observed with increasing fluoride applications (Austin *et al.* 2010; Maggio *et al.* 2010). High fluoride concentrations have also been observed to reduce erosive wear. Pre-application of stannous and sodium solutions at 9,500ppm fluoride were unable to offer protection after 9 cycles of severe erosive challenges (2 min 0.01M HCL, pH 2.2) whereas a protective effect was observed with a sodium fluoride varnish at 42,500ppm fluoride (Austin *et al.* 2011). However, this may also be due to the tribology at the dental surface and the increased adherence of the varnish to the surface. A study investigating equal concentrations of titanium fluoride varnish or solution prior to an erosive cycling model, observed significantly decreased wear with the varnish compared to the solution (Magalhães *et al.* 2008).

Acidified fluoride has also been observed to result in decreased wear (Wiegand, Magalhães, *et al.* 2009). The low pH of the medium encourages low levels of demineralisation of surface hydroxyapatite which facilitates the formation of fluoroapatite on the surface (Attin *et al.* 1999; Larsen and Richards 2001).

Few epidemiological studies have investigated the role of fluoride in the prevention of erosive wear. A large study on 2,456 Irish adults observed no significant relationship between exposure to water fluoridation and tooth wear (Burke *et al.* 2010). This contrasted with an epidemiological study carried out in the UK on 2,351 14 year olds where exposure to water fluoridation was associated with less exposed dentine on buccal and palatal surfaces of assessed teeth. No differences were observed for the occlusal/incisal surfaces. The same study observed a protective effect when teeth were brushed with a fluoride toothpaste twice daily (Bardsley *et al.* 2004).

There is difficulty in isolating the protective mechanism of action of fluorides as erosion experiments often involve cycling of the fluoride and erosion. The presence of calcium fluoride (CaF_2) and sacrificial intraoral fluoride ions, remineralisation of the eroded structure, or fluoroapatite formation inhibiting demineralisation, all may occur in the cycling process. Reviews suggest that all three mechanisms can be effective in mild erosive challenges, particularly when paired with metal cations (Huysmans *et al.* 2014). However this role may be limited under severe or repeated erosive challenges (Austin *et al.* 2010; Austin *et al.* 2014; Ganss *et al.* 2015). The clinical relevance of the degree to which remineralisation can occur is also under debate (Lussi *et al.* 2014).

1.2.3.1.2 Different types of fluoride

There is substantial laboratory evidence that metal cations, particularly stannous fluoride and titanium tetrafluoride, can play a role in protection against erosive wear (Wiegand, Bichsel, *et al.* 2009; Stenhagen *et al.* 2013). Recent literature has focused on the stannous ion (Huysmans *et al.* 2014). In contrast to the monovalent sodium cation which is incapable of forming complex deposits, pre-treatment with

stannous fluoride (Schlueter, Klimek, *et al.* 2009b) and titanium tetrafluoride (Magalhães *et al.* 2008) can form layers of metal deposits on the surface of enamel in the laboratory. This may act as a physical barrier inhibiting acid contact (Schlueter, Klimek, *et al.* 2009b).

The increased focus on stannous fluoride in erosive wear research is predominantly due to two factors. Stannous fluoride formulations require a native acidic pH as neutral solutions are not stable (Faller *et al.* 2014). Similar to the mechanism of action of acidified gels, this releases ions from the dental surface allowing penetration of the fluoride ion and formation of fluoroapatite (Schlueter, Klimek, *et al.* 2009b). The stannous ion also has the same valency of the calcium ion and has been observed to directly remineralise hydroxyapatite structure (Schlueter, Hardt, *et al.* 2009; Ganss, Hardt, *et al.* 2010). One in vitro study observed complete inhibition of enamel erosion when stannous fluoride was applied immediately after a citric acid challenge (Ganss *et al.* 2008).

Amine fluoride has also been frequently investigated. Some studies have found it to be more protective than sodium fluoride (Wiegand, Bichsel, *et al.* 2009) with others observing it to have similar protective effects (Ganss *et al.* 2008; Faller *et al.* 2014). The calcium ion has also been investigated in an attempt to reduce erosive tooth wear. A recent study observed that although remineralisation of enamel occurred more rapidly with a sodium fluoride dentifrice, enamel remineralised with calcium was less susceptible to further demineralisation (Pignatelli *et al.* 2016). Other authors have reported that calcium products also showed remineralising potential (Ranjitkar *et al.* 2009; Carvalho *et al.* 2013) with an enhanced effect when combined with fluoride (Srinivasan *et al.* 2010). In contrast,

some authors failed to observe protective benefits with the calcium ion over a negative control (Wegehaupt and Attin 2010; Wiegand and Attin 2014).

These studies rely on the assumption that polished and flat enamel surfaces respond to acids in a similar way to natural surfaces either in the laboratory or clinically. To date there is little information to understand how fluoride responds to erosion on natural tooth surfaces and more work is needed in this area.

1.2.3.1.3 Timing of fluoride application

The ideal timing of fluoride application essentially questions whether the fluoride compound is more effective at preventing demineralisation (surface protection) or encouraging remineralisation (surface re-hardening). The unique properties of each fluoride may suggest that they may be optimally applied at different times in relation to the acid challenge. Some authors have applied sodium fluoride before the erosive challenge and found little to no effect (Wiegand, Bichsel, *et al.* 2009; Hystad Hove *et al.* 2014). Whereas other authors have applied sodium fluoride after erosion and found a protective effect (Comar *et al.* 2012; Mathews *et al.* 2012).

Only one study, to the authors knowledge, investigated the effects of rinsing before an erosive challenge compared to rinsing after, using an amine fluoride solution (Lussi, Jaeggi, Gerber, *et al.* 2004). Rinsing with amine fluoride after the erosive challenge produced the least wear although it was observed that both did not reduce subsequent toothbrush abrasion (Lussi, Jaeggi, Gerber, *et al.* 2004). This may not be true for other metal ions with differing mechanisms of action. The stannous ion shows promising results in the prevention of dental erosion, either combined with fluoride or in the form of other stannous salts (Schlueter *et al.*

2010). Interestingly, there are indications that stannous deposits are more stable on dental surfaces than sodium fluoride deposits when facing an erosive challenge (Khambe *et al.* 2014). The lower pH of stannous fluoride upon application may also be more effective when placed into a neutral environment compared to sodium fluoride which is more effective when acidified. Further research is required into the optimal timing of fluoride application in the prevention of erosive tooth wear progression.

1.2.3.2 Tooth brushing and erosive wear

The positive association between fluoride application and erosive tooth wear becomes complicated when the abrasive wear action of tooth brushing is taken into consideration. Tooth brushing in a neutral pH, with a normal brushing force and a low abrasive toothpaste results in a negligible amount of wear (Wiegand *et al.* 2007). However toothpastes with high relative dentine abrasivity/relative enamel abrasivity (RDA/REA) can produce substantial wear (Joiner *et al.* 2004). Filament stiffness has also been implicated with recent studies observing soft toothbrushes to retain dentifrice and as a result, increase abrasive wear (Bizhang *et al.* 2016). The pathological interaction between erosion and abrasion is particularly synergistic as softened dental tissues are susceptible to mechanical forces (Mair 2000). This has led to ambiguity regarding the ideal timing of tooth brushing in relation to an acidic challenge.

1.2.3.2.1 Timing of toothbrushing and salivary remineralisation

As discussed in section 1.3.2, saliva has been thought to remineralise eroded enamel. It has been hypothesised that allowing a period of salivary remineralisation following an erosive challenge will reduce susceptibility to tooth brush abrasion (Jaeggi and Lussi 1999; Amaechi and Higham 2001; Attin *et al.* 2001). Subsequent recommendations to delay tooth brushing for periods of up to one hour after eating are based upon relatively old laboratory investigations. Jaeggi & Lussi 1999 observed statistically reduced abrasive wear in situ when eroded specimens were retained in the mouth for one hour prior to brushing (Jaeggi and Lussi 1999). Attin *et al.* subjected specimens to mild erosive challenges in vitro (Attin *et al.* 2000) and in situ (Attin *et al.* 2001), performing abrasion at various intervals. The authors observed a linear relationship between remineralisation period and reduced abrasive wear. Figure 3 compares the data from the experiments. Although different cycling regimes were observed (the in vitro trial consisted of 10 cycles of 60 s exposures to Sprite light and artificial saliva with no proteins, compared to the in situ with 21 days of erosive cycling with 90 s exposures to Sprite light), relatively small effect sizes were observed with large standard deviations, particularly in situ.

Figure 3: Profilometric data from experiments performed by Attin *et al.* 2000 and Attin *et al.* 2001

Remineralisation Period	Profilometric loss in vitro (Attin <i>et al.</i> 2000)	Profilometric loss in situ (Attin <i>et al.</i> 2001)
0 min	5.16±1.26 µm	6.78 ± 2.71 µm
10 min	2.47±0.68 µm	5.47 ± 3.39 µm
60 min	1.72±0.75 µm	4.78 ± 2.57 µm
No abrasion	0.81±0.23 µm	0.66 ± 1.11 µm

The authors suggested that clinical erosive wear could be limited by observing a waiting period after an erosive challenge. However, recent studies have observed no statistical reduction in erosive/abrasive wear after 2 hours in situ (Ganss, Schlueter, et al. 2007) and 4 hours in situ (Lussi *et al.* 2014). These authors advised reconsideration of guidelines to wait for one hour after brushing.

Two studies to the author's knowledge have compared brushing before an erosive challenge to brushing after an erosive challenge in situ. Wiegand *et al.* 2008 prepared both enamel and dentine specimens which were then either brushed, exposed to saliva for 5 minutes and then eroded or, for the second group, eroded, exposed to saliva for 5 minutes and brushed. Brushing before the erosive challenge resulted in less tooth wear (Wiegand *et al.* 2008). Unfortunately flaws in the experiment design render it difficult to gauge whether the protective effect was a result of the acquired pellicle, which was only present in the first group, or the timing of brushing. There was also no fluoride application in the experimental design. Ganss *et al.* 2007 observed no statistical difference in wear, when enamel specimens were brushed before or immediately after an erosive challenge in the presence of a salivary pellicle. Again, there was no fluoride application in this part of the in situ study design (Ganss *et al.* 2007).

1.2.3.2.2 Timing of toothbrushing and fluoride therapy

This relationship is complicated further when the protective role of fluoride is taken into consideration. One must consider whether the benefits of fluoride application when brushing outweigh the risks of abrasive wear. One cross-over in situ study lasting 5 days, observed that brushing with a stannous fluoride

toothpaste immediately after an erosive challenge was not statistically different from erosion only with no abrasion (Ganss, Schlueter, *et al.* 2007). Another study investigating application of toothpaste slurries before an erosive challenge compared to application of the slurries after observed that application of toothpastes before the erosive challenge resulted in less surface hardness change with no differences between the different tooth paste formulations (Lussi *et al.* 2008). However there was no abrasive element to this study design.

An unusual in situ study design asked participants to brush their teeth prior to inserting eroded specimens to assess the remineralisation potential of intraoral fluoride reservoirs from brushing (Magalhães *et al.*). No statistical differences were noted in those who had brushed their teeth prior to specimen insertion compared to those that had not.

Again, there is a paucity of epidemiological studies investigating the relationship between timing of toothbrushing in relation to mealtimes and erosive wear. One large multi-centre epidemiological study on 3,187 participants observed no relationship between erosive wear and brushing teeth immediately after breakfast. In contrast, an increased relationship with tooth wear was observed when tooth brushing was delayed by up to 44 minutes (OR up to 1.41 [95% CI 1.07-1.86]) (Bartlett *et al.* 2013). Based upon these findings, the authors advised that dentists should not advise patients to delay brushing after breakfast.

Multiple studies have investigated the relationship between frequency of tooth brushing and erosive wear. Although there have been studies that have reported increased erosive wear with increased frequency of brushing (Lussi and Schaffner 2000; Alvarez Loureiro *et al.* 2015), other studies have shown increased risk when

brushing was performed less than twice daily (Mulic *et al.* 2012; Hasselkvist *et al.* 2014; Zhang *et al.* 2015; Sovik *et al.* 2015; Teixeira *et al.* 2016). This again may be due to the protective action of fluoride (Bardsley *et al.* 2004). Relatively little is known about the clinical impact of brushing immediately after an erosive challenge.

1.3 DIET ASSESSMENT, ADVICE AND BEHAVIOUR CHANGE IN A CLINICAL SETTING

1.3.1 ASSESSING THE DIET

1.3.1.1 Dietary assessment methods

Diet records, 24-hour recall and food frequency questionnaires (FFQ's) are the most common forms of dietary assessment for epidemiological studies (Shim *et al.* 2014). Diet records involve recording each food item prior to consumption over a given time period, typically 3-5 days. This minimises reliance on the respondents' memory however it requires continuous motivation of the participant. In addition, participants have been observed to alter their diet intentionally during the period of observation or deliberately not report intakes (Margetts and Nelson 1997). 24-hour recall is when the interviewer asks the respondent to remember in detail everything consumed in the previous 24 hours. It relies on accurate memory of intake and may be helped by the interviewer prompting the respondent to remember eating and drinking episodes by time periods e.g. breakfast, mid-morning snack (Shim *et al.* 2014). The primary limitation is that recording consumption for a single day is seldom representative of individuals intake (Cade *et al.* 2002).

Both of these methods focus on short term intake as opposed to long term exposure although it is accepted that one is moderately correlated with the other (Margetts and Nelson 1997). FFQ's ask the respondent how often they consume

items over a defined period. They predominantly consist of lists of food/beverages and a selection of options relating to frequency. FFQ's are designed to capture habitual intake and collect information from large levels of respondents. Many FFQ's attempt to collect information about portion size or quantity and may be referred to as semi-quantitative FFQ's (Shim *et al.* 2014). Typically FFQ's measure intake by assigning a single daily intake as the baseline and evaluate less frequent or more frequent intakes as proportions of this. For example a single intake once a week would be 0.14 or one seventh of an intake, an intake twice a week would be 0.29 or two sevenths of an intake (Okunseri *et al.* 2015). FFQ's have been observed to be relatively poor at detecting weak associations, tend to be less specific and may have greater measurement error (Schatzkin *et al.* 2003). However, there is evidence to suggest that, provided they are sufficiently validated and specific to the item of interest, they can accurately measure the area of interest (Cade *et al.* 2004; Subar 2004). Food frequency questionnaires have been commonly used in erosion and caries research (Mulic *et al.* 2012; Bartlett *et al.* 2013; Hasselkvist *et al.* 2016). Diet records incorporating a weekend have also been recommended to estimate the daily acid challenge (Lussi and Hellwig 2014). No method is ideal; all are an estimation of the diet and all are subject to bias.

1.3.1.2 Limitations of dietary assessment

Reporting error is introduced when individuals are relied on to accurately and honestly report intake (Wiren *et al.* 2003). The foods standards agency (FSA) in Scotland investigated the problem of underreporting in dietary assessment methodology (Wiren *et al.* 2003). When reviewing the literature, it was observed the two types of bias to be introduced were observer bias (participants changing eating behaviour as they knew they were being observed) and

misreporting their eating behaviour, particularly with “socially undesirable” foods/beverages.

Diet *also* changes over time and eating habits reported now are not always indicative of past dietary history. This is particularly relevant for dental erosion as it is very difficult to gauge periods of active disease from inactive disease (Bartlett 2003). One erosion study attempted to include any changes that had occurred over a two month period (Bartlett, Fares, *et al.* 2011). The authors concluded that the time interval was too short and no statistical differences were observed. In contrast, relying on memory to accurately assess participants past intake after a change in diet has occurred is difficult (Margetts and Nelson 1997). There is evidence however, to suggest that data obtained from episodic recall (recall of frequency of a behaviour that was performed on a regular basis for a prolonged period of time) is more reliable (Menon 1993).

1.3.1.3 Interviewer-led questionnaires vs. self-completed questionnaires

There is a capacity to reduce bias through the mode of questionnaire

administration which can directly impact on the quality of the data (Bowling 2005). The majority of dietary questionnaires typically used in erosion studies have been self-administered questionnaires (Dugmore and Rock 2004a; El Aidi *et al.* 2011; Bartlett *et al.* 2013). Self-administered questionnaires have advantages; they are cheaper and as a result, have potential to be given to a greater number of participants. They also have less potential for social desirability bias and participants may be more willing to disclose sensitive information (Bowling 2005). However, the multi-factorial nature of erosive tooth wear poses challenges when attempting to capture a comprehensive risk pattern from a patient. It is important that the participant fully understands the questions being asked, is able to clarify

what aspects of the diet are acidic and can report fully on each risk factor. Self-administered questionnaires also do not allow the freedom to confirm the answers of the respondents and give clarification to respondents. A meta-analysis reported that a greater amount of information was given by respondents in a face-to-face interview (De Leeuw and Van der Zouwen 1988). They observed that a motivated interviewer could increase question response rates, maintain motivation with more difficult questions, probe for responses, clarify ambiguous questions on the spot, aid with recall of events and behaviour and ensure mutual understanding of the question and answer had occurred (De Leeuw and Van der Zouwen 1988). The use of interviewers also allows for immediate checking by the interviewer of improbable or unlikely responses. The ability to add open-ended questions can increase the amount of information collected and the questionnaire can be applied to a diverse group with a range of eating/drinking habits (Cade *et al.* 2002).

Disadvantages of interviewer-led questionnaires include the need for interviewer standardisation and increased cost. Social desirability bias is also increased through the presence of an interviewer (Schnell and Kreuter 2005). This can be enhanced in a clinical setting whereby there are obvious interviewer characteristics, i.e. a dental professional, and often a clear, socially desirable answer (Tourangeau and Yan 2007). Factual items, non-sensitive items, easily comprehended items and closed-question items have all been shown to be less vulnerable to interviewer effects (Schnell and Kreuter 2005; Tourangeau and Yan 2007). Interviewer effects can be reduced if the interviewer has received good training and with the use of a standardized procedure including neutral wording,

neutral probing and a non-judgemental repertoire (Schnell and Kreuter 2005; Tourangeau and Yan 2007).

1.3.2 PROVIDING DIETARY ADVICE

1.3.2.1 Current dietary advice targeting erosive tooth wear

The first comprehensive published preventive guidelines to prevent dental erosion were based upon theoretical laboratory mechanisms (Imfeld 1996b). As our knowledge of the pathological process of erosive tooth wear increases many recommendations are undergoing academic debate. For example, Imfeld recommended rinsing with water after acid consumption. To the author's knowledge, no studies have observed a protective effect with rinsing with water. In contrast, this may increase the erosive wear process by disrupting the equilibrium present on teeth and clearing remineralising ions from the oral environment (Mistry *et al.* 2015). Imfeld also recommended a soft or medium toothbrush to be used by the patient. However, recent scientific reports suggest they may be associated with increased abrasive wear (Bizhang *et al.* 2016). Furthermore, Imfeld recommended that occlusal restorations be placed to prevent the loss of the occlusal vertical dimension. Recent guidelines suggest delaying treatment until the underlying cause is addressed (Bartlett *et al.* 2008).

Figure 4 reports two examples, one with a dietician as a co-author (Auad and Moynihan 2007) and the other, written by experts in the field of erosion (Lussi, Jaeggi, and Zero 2004).

Figure 4: Table comparing dental dietary advice provided by a dietitian and dental erosion experts.

Auad & Moynihan 2007	Lussi <i>et al.</i> 2004
<ul style="list-style-type: none"> • Reduce the frequency and amount of consumption of acidic drinks and foods, and especially discourage the consumption of acidic drinks at bedtime. • Encourage the consumption of water and nutritious beverages such as milk, and also the consumption of fresh fruits, when part of a healthy and balanced diet. Recommend the consumption of a neutralizing food, such as cheese, after the intake of an acidic drink or food. • Recommend that acidic drinks not be added to infant feeding bottles. • Suggest that if soft drinks are consumed, they should preferably be chilled, consumed in one sitting, and limited to mealtimes. • Discourage the consumption of acidic sweets, especially between meals. 	<ul style="list-style-type: none"> • Reduce acid exposure by reducing the frequency (main meals only), and contact time of acids. Do not hold or swish drinks in your mouth. • Finish meal with something (rich in $\text{Ca}^{2+}/\text{PO}_4^{3-}$) 'neutralising' acidic food such as cheese. After acid intake stimulate saliva flow with chewing gum. • Avoid toothbrushing immediately after acid intake. Instead, rinse with fluoride containing mouthrinse or with water. • Apply fluoride before the erosive challenge Use high concentrated topical fluoride periodically.

Overall, there is no one clear consensus on preventive advice. Some experts offer advice targeting all aspects of erosive wear (Bartlett 2005) and others are specifically targeted at the diet (Lussi, Jaeggi, and Zero 2004; Auad and Moynihan 2007). These reviews/opinion articles convey similar messages to reduce frequency of acid consumption, limit dietary acids to mealtimes/consume alongside a neutralising food and to use fluoride oral hygiene products. Advice regarding timing of brushing in relation to acid intake is ambiguous. Although the

underlying theory is sound, few of these recommendations, apart from reducing acid consumption and the daily use of fluoride products, are supported by robust clinical data.

1.3.2.2 Diet advice and behaviour change

When dietary advice is provided, it may not be effective at inducing a behaviour change (Ashenden *et al.* 1997). One longitudinal study investigating tooth wear progression over 6 years observed that dietary behaviour had not changed despite being provided with “extensive dietary counselling” (Lussi and Schaffner 2000). A Cochrane review investigating dietary advice provision in dental practice suggested there is evidence, albeit limited, that one-to-one dietary advice interventions can change behaviour (Harris *et al.* 2012). The authors reported a positive association between diet advice and behaviour change. The evidence was classified as weak, due to the lack of well-designed reported studies. The authors also noted that no studies meeting the inclusion criteria investigated dietary erosive wear.

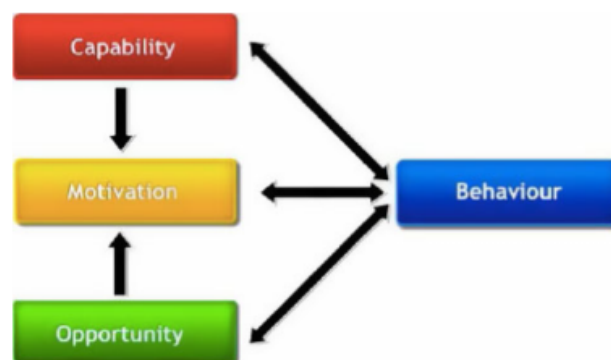
Harris *et al.* described three different types of dietary advice in the literature (Harris *et al.* 2012).

1. Health education: educating patients to change their knowledge.
2. Health advice: giving health advice and supporting lifestyle change.
3. Undertaking behavioural interventions: using behavioural strategies, specifying the changes to be made, relapse prevention, identifying barriers to change etc.

There has been a recent drive to look at behavioural interventions to address the seeming gap between advice and adherence to advice (Watt *et al.* 2003; Michie *et*

al. 2013). A systematic review observed that behaviour change interventions tend to be more successful when established behaviour change techniques are utilised compared to dietary advice alone (Greaves *et al.* 2011). This was independent of the intervention provider, the setting of the intervention or the study population (Greaves *et al.* 2011). For dietary advice to result in a behaviour change, the advice needs to be specific, the patient needs to recall the advice at the appropriate time, following which, the patient needs to act on the advice (Watt *et al.* 2003). The COM-B model, adapted from Michie *et al.* (Michie *et al.* 2015), was proposed by Asimakopoulou and Newton as a tool to help choose effective behaviour change techniques within a dental setting (Asimakopoulou and Newton 2015).

Figure 5: COM-B Model proposed by Asimakopoulou and Newton 2015 for use within a dental setting.



This model is based upon the principle that in order to achieve behaviour change, the person must have the capability to perform the behaviour change (i.e. the knowledge and skills necessary), the opportunity to change (i.e. the environmental context and resources available are suitable) and the motivation to change (positive intentions and beliefs). Once the areas to target have been identified, a behaviour change technique can then be more effectively chosen. Figure 6 reports

a summarised list compiled by Asimakopoulou and Newton 2015 adapted from Michie *et al.* 2015)

Figure 6: Summary of behaviour change techniques with examples (Asimakopoulou & Newton 2015)

Behaviour change technique cluster	Examples of specific techniques defining the cluster
1. Scheduled consequences	Punishment, extinction, shaping, negative reinforcement and differential reinforcement
2. Reward and threat	Social, material or self-reward, nonspecific reward, anticipation of future rewards or removal of punishment and threat
3. Repetition and substitution	Habit reversal or formation, graded tasks and behavioural rehearsal/practice
4. Antecedents	Restructuring the physical or social environment, avoidance or changing exposure to cues for the behaviour
5. Associations	Classical conditioning, cues and discriminative cue
6. Covert learning	Vicarious reinforcement and covert conditioning
7. Natural consequences	Health, social, emotional consequences and salience of consequences
8. Feedback and monitoring	Biofeedback, feedback on behaviour and self-monitoring of behaviour
9. Goals and planning	Action planning, problem/coping planning goal setting, behavioural contract, review behaviour or outcome goal
10. Social support	Practical, general and emotional social support
11. Comparison of behaviour	Modelling, social comparison and information about others' approval
12. Self-belief	Focus on past successes and mental rehearsal of successful performance
13. Comparison of outcomes	Pros and cons, persuasive argument, comparative imagining of future outcomes
14. Identity	Self-affirmation, identification of self as role model, cognitive dissonance and reframing
15. Shaping knowledge	Behavioural experiments, antecedents and reattribution,
16. Regulation	Regulate negative emotions, pharmacological support and conserving mental resources

While motivation may be important when setting a goal, other factors may be more important when adhering to the targeted behaviour (Gollwitzer and Sheeran 2006). One study assessed adherence to flossing behaviour, recording baseline levels of motivation after a self-monitoring behaviour change intervention was performed. Those that self-monitored were observed to have reduced plaque scores and bleeding scores compared to those that did not receive the intervention, regardless of motivational stage (Suresh *et al.* 2012). This was supported by a meta-analysis which observed that opportunity related factors, specifically heightened accessibility of the opportunity and a strong response link to it, were observed to have the strongest association with behaviour change rather than level of deliberation (Webb and Sheeran 2008). A meta-analysis investigating different interventions in health-related behaviours identified

implementation planning as a promising behaviour change technique (Gollwitzer and Sheeran 2006). Implementation planning is the process of planning the intended behaviour in advance while anticipating obstacles/barriers. One form of this is “if-then planning” which aims to prompt the patient with a situational cue reminding them of their new intention. For example, “if I want to go outside for a cigarette then I will go for a walk instead” (an if-then plan). This method, applied within a dental setting, has been observed to statistically improve flossing behaviours compared to flossing advice alone (Schüz *et al.* 2006; Schüz *et al.* 2009). Outside of dentistry, this method has been successfully used to increase physical activity in the obese (Olander *et al.* 2013) and elderly (French *et al.* 2014). Although there is insufficient evidence to recommend that they be applied routinely (Werner *et al.* 2016), behavioural change techniques show promise to be applied within a dental clinical setting (Asimakopoulou and Newton 2015). The lack of rigorous, well-designed behavioural intervention research represents a barrier to research in this field (Lorencatto *et al.* 2013) and multiple reviews have called for higher quality research investigating behavioural interventions within a dental setting (Renz *et al.* 2007; Harris *et al.* 2012; Adair *et al.* 2013; Newton and Asimakopoulou 2015; Werner *et al.* 2016).

1.4 MEASURING EROSIVE TOOTH WEAR

1.4.1 CLINICAL INDICES

There are a multitude of indices reported in the literature aiming to assess erosive tooth wear (Bardsley 2008). Figure 7 provides a brief overview. Eccles first proposed an index to assess erosion of non-industrial origin in 1979 (Eccles 1979). Although the actual index itself is quite brief, the descriptions given in the text are relatively complicated and focused solely on dental erosion. Smith and Knight proposed the Tooth Wear Index (TWI) in 1984, increasing the detail on different dental surfaces and grading tooth wear regardless of aetiology. This index and subsequent modifications have been the most commonly used tooth wear index in epidemiological studies (Dugmore and Rock 2003b; Bartlett, Fares, *et al.* 2011; Okunseri *et al.* 2015). Intra- and inter- examiner reproducibility were within acceptable ranges for epidemiological purposes, despite some authors arguing that the level of detail resulted in poorer inter-examiner correlations and time-consuming examinations (Larsen *et al.* 2000). In contrast, Donachie and Walls recommended an increase in detail and number of classifications after criticising the index for being too insensitive for an aging population and unable to distinguish between pathological and physiological wear in the elderly (Donachie and Walls 1996).

Lussi recommended simplifying the index in 1996, classifying wear by level of dentine exposure (Lussi 1996). However, this was criticised as erosive wear can be quite severe without penetrating into dentine (Ganss and Lussi 2008).

Furthermore, a laboratory study observed that clinical diagnosis of dentine exposure may not be a reflection of the histological diagnosis (Ganss *et al.* 2006).

The Basic Erosive Wear Examination (BEWE) was developed by expert consensus in 2008 (Bartlett *et al.* 2008) and appears to be increasingly adopted (Bartlett 2016a). It is a simple scoring system quantifying the size of erosive lesions as a percentage of the surface affected. It does not distinguish between enamel and dentine although it does highlight that in scores 2 and 3 dentine is often involved. The paper also advocated summing the maximum score in each sextant to assess treatment need. However, this score is not scientifically validated and is based upon expert opinion of one clinician (Bartlett *et al.* 2008). Full mouth scores must also be interpreted with caution as there is potential to mask localised areas of severe tooth wear. Investigations comparing the different indices as a screening tool have reported satisfactory examiner reliability with the BEWE (Mulic *et al.* 2010; Margaritis *et al.* 2011; Dixon *et al.* 2012). There remains a conflict between a useful and practical index for epidemiological studies and a clinical index to measure tooth wear progression (Ganss and Lussi 2008). The Exact Tooth Wear Index was designed to provide a more sensitive index to monitor progression (Fares *et al.* 2009) with five grades of wear for each of enamel and dentine. All teeth are graded by percentage of the surface area affected by erosion at 10%, 33% and 66% levels. This index was used by Harding *et al.* in their longitudinal study on 123 children (Harding *et al.* 2010) and Bartlett *et al.* in a study on 1,010 university students (Bartlett, Fares, *et al.* 2011). However it has not been widely utilised by other research groups. A recent meta-analysis reported that erosion prevalence rates could vary depending on the detail of the index used (Salas *et al.* 2014). Whereas some indices may be highly sensitive at measuring the incidence/prevalence of erosive tooth wear with good inter-examiner reliability, others may not be sufficiently detailed to monitor progression as discussed in the following section.

Figure 7: Simplified versions of commonly used indices for comparability

	Eccles index 1979	Smith and Knight Tooth Wear Index (TWI) 1984	Lussi Index 1996	Basic Erosive Wear Examination (BEWE) 2008
0		No loss of enamel surface characteristics or contour	No erosion, smooth, silky-shining appearance, absence of developmental ridges possible	No erosive tooth wear
1	Superficial lesions – involving enamel only	Loss of enamel characteristics Loss of contour cervically	Loss of surface enamel but no dentine exposure, width of lesion exceeds its depth	Initial loss of surface texture
2	Localised lesions – dentine exposure <1/3 of surface	Dentine exposure <1/3 of surface. Mild dentine exposure incisally. Cervical lesion <1mm deep	Dentine exposed < 50% of surface	Distinct defect, hard tissue loss <50% of surface area
3	Generalised lesions – dentine exposure >1/3 of surface a) Facial surfaces b) Lingual and palatal surfaces c) Incisal and occlusal surfaces d) Severe multi-surface involvement	Dentine exposure >1/3 on occlusal/buccal/lingual surfaces. Loss of enamel and substantial loss of dentine incisally. Cervical defect 1-2mm deep	Dentine exposed >50% of surface	Hard tissue loss >50% of surface area
4		Complete enamel loss, pulp exposure or secondary dentine exposure		

1.4.2 LONGITUDINAL SUBJECTIVE MEASUREMENT OF TOOTH WEAR

Subjective longitudinal measuring of erosive tooth wear has been attempted using clinical indices alone (Dugmore and Rock 2003b) or with the use of study casts (Ganss *et al.* 2001). Comparing study casts taken at separate intervals over time is often recommended as a method to monitor progression of erosive wear clinically (Bartlett *et al.* 2008). The advantage of using casts is evaluation can be performed repeatedly, by multiple examiners, under ideal viewing conditions and with assessment of the occlusal relationship if necessary. Although this form of monitoring is very accessible it relies on long term collaboration between the patient and their dentist, is highly subjective and cannot monitor progression over a short period of time. Furthermore, a compliance rate of 34% was reported in an audit of GDP's who were recommended by restorative consultants to take study casts to monitor wear (Bartlett *et al.* 2005). There are also limitations to assessment on study models. The optical properties and surface characteristics of enamel cannot be assessed which makes the diagnosis of early smooth surface lesions difficult (Johansson *et al.* 2002). The exposure of dentine, an important assessment criteria for many of the clinical indices, cannot be accurately assessed on study casts (Johansson *et al.* 1993; Larsen *et al.* 2000). Inter- and intra-examiner reliability tend to be higher when a clinical assessment is performed as opposed to assessment on study casts (Wetselaar *et al.* 2009; Mulic *et al.* 2010; Hove *et al.* 2013). Authors have argued that clinical photographs have the same level of accuracy at detecting tooth wear as study models (Larsen *et al.* 2000; Mulic *et al.* 2010; Hove *et al.* 2013) although no longitudinal studies utilising this method have been done to date.

Orthodontic casts have been used to assess tooth wear over longer time periods up to 20 years (Knight *et al.* 1997). The main limitation of this technique is that the aetiology cannot be reported in conjunction with progression. Ganss *et al.* 2001 followed 265 participants over 5 years observing statistical changes over that period (Ganss *et al.* 2001). Vervoorn-Vis *et al.* assessed the orthodontic study casts of 40 patients at three time intervals over 9 years and were able to detect significant differences at 4 and 5 year intervals (Vervoorn-Vis *et al.* 2015). Both authors concluded that given the slow rate of tooth wear, monitoring with study casts should be done over equivalent time periods of 4-5 years.

Furthermore, the sensitivity of clinical monitoring over short periods has yet to be established. Direct comparisons between studies are difficult as different indices are more sensitive than others. For instance, the Smith and Knight index calculates wear at the 33% and 66% levels whilst the BEWE is at 50% and the latter does not assess dentine exposure. Despite this, clinical examinations using indices may be more sensitive when measuring wear progression over a shorter period than evaluation of study casts. El Aidi was able to observe statistical differences at 18 months using indices clinically (Aidi *et al.* 2011) whereas Johansson *et al.* 1993 was unable to detect statistical differences at 18 months using study models (Johansson *et al.* 1993). Dugmore and Rock observed using clinical examinations that wear progressed in 26.8% of participants over 2 years (Dugmore and Rock 2003b) whereas Bartlett observed mild tooth wear progression on relatively few surfaces when assessing orthodontic models over the same time period (Bartlett 2003). A 7 year longitudinal study on children used a modified form of the Smith and Knight Index to detect wear on 38% of participants (n=123). Although the

authors did not report the percentage of participants with wear progression, they observed that presence of wear in the permanent dentition was associated with an increased odds ratio of 5.06 (95% CI 1.32 – 19.39) when presence of wear in the primary dentition was detected (Harding *et al.* 2010).

There are three studies to the author's knowledge that performed quantitative assessment of erosive tooth wear progression in addition to grading using indices on casts. In the majority of cases, erosive damage was subclinical over a time period of 1 year to 18 months (Chadwick *et al.* 2005; Al-Omiri *et al.* 2010; Rodriguez *et al.* 2012a; Al-Omiri *et al.* 2013). Al-Omiri observed that the Smith and Wear Index was unable to monitor tooth wear over a 6 months and 1 year (Al-Omiri *et al.* 2010; Al-Omiri *et al.* 2013). Chadwick *et al.* did not observe visual differences after 18 months using a Ryge index (Chadwick *et al.* 2005) and Rodriguez *et al.* did not observe statistical clinical difference on study casts using indices over a 1 year period (Rodriguez *et al.* 2012a).

To conclude, although direct clinical observations using indices may detect wear over an 18-month period, there are no reports that the use of indices on study casts can detect changes over less than an 18-month period. Regardless of the index used and whether assessment is performed on patients or casts, there is a degree of subjectivity. The resolution of a healthy eye under ideal focus conditions at a distance of 22cm is an average of 75-100 μm (Gross *et al.* 2008). Rodriguez *et al.* reported that over 70% of participants in the study (total n=63) had tooth wear progression $<15 \mu\text{m}$ over a 6 month period (Rodriguez *et al.* 2012a). To detect changes when monitoring over a short time period, evidence would suggest that laboratory equipment is needed.

1.4.3 LONGITUDINAL QUANTITATIVE MEASUREMENT OF TOOTH WEAR

The difficulty with the use of quantitative laboratory techniques is the lack of a fixed intraoral reference point. Two predominant methods used by research groups to attempt to overcome this problem are constructed fixed intraoral reference points discussed in section 1.5.3.1 and surface matching software discussed in section 1.5.3.2.

1.4.3.1 Intraoral reference points

Molnar *et al.* 1983 was the first to attempt to define a reference point when monitoring attritive tooth wear. Attritive wear was investigated in a longitudinal study on Australian Aborigines (n=64) from the age of 7 to 18 years. Using a depth gauge, the deepest part of the central pit was used as a reference point to measure wear of the cusp apices. Interestingly, when looking at intraoral photographs taken from the study there is a clear erosive element as defined by the loss of enamel characteristics and early stages of cupping on the cuspal tips of the canine, premolar and first molar. Despite the fact that the reference point was also getting worn (see figure 8 below) reduction in crown height was reported at 41 μm per year.

Figure 8: Figure taken from Molnar *et al.* 1980. These are not taken from the same patient.

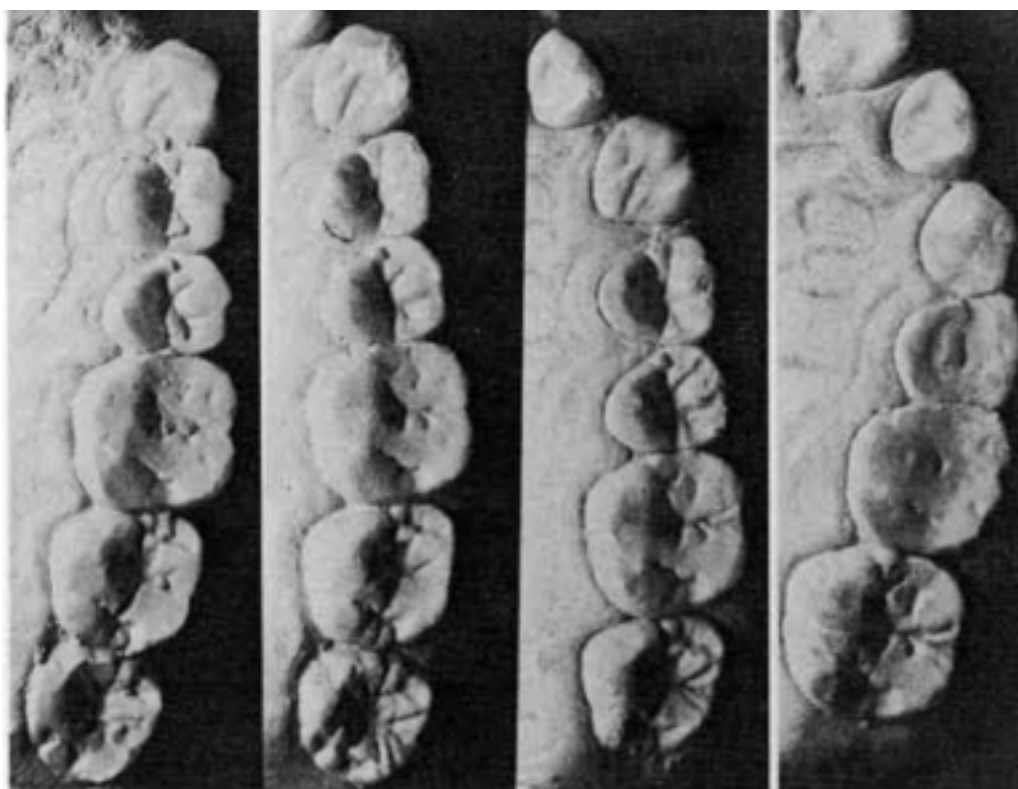


Fig. 3 — Progress of dental wear of a male Aborigine. Four stages of wear are shown in these photos of casts of the left maxillary arches at ages seven, 12, 18, and 25.

Bartlett *et al.* 1997 attempted to create an intraoral reference by cementing custom-made metal disks to the palatal surface of upper incisors in a group with erosive wear and a control group. Impressions were taken at baseline and at 6 months and scanned using a non-contacting laser profilometer. Step height change from the top of the disc over 6 months was estimated to be a median of 36.5 μm (range 17.6-108.2) for participants with erosive wear and 3.7 μm (range 0.5-15.8) for controls. The same research group used similar methods to estimate the protective effect of dentine bonding agents (Sundaram *et al.* 2007) and fissure sealants (Bartlett, Sundaram, *et al.* 2011). However, sample size difficulties were faced when there were issues with retention of the metal disks. Schlueter *et al.* 2005 argued that although the methodology showed promise the disk shape was

too undefined to get repeatable measurements (Schlueter *et al.* 2005). Their group attempted to refine this method by using stars instead of disks and measuring the tip of each star giving five readings. It was validated in vitro with an estimated accuracy of the process to be 15 μm (Schlueter *et al.* 2005). However, the research group has not employed this method in vivo to date.

1.4.3.2 Surface matching software

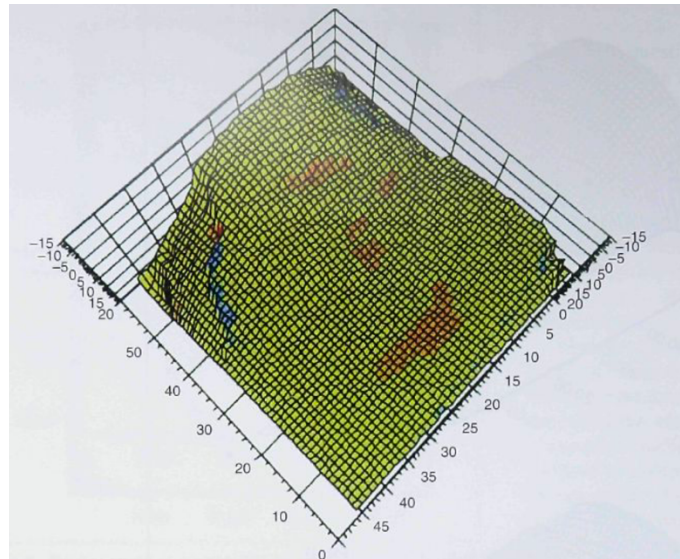
The use of surface matching software removes the need for an intraoral reference point by aligning digitised scans of test surfaces calculating differences between them. The software has either been manufactured by the university (DeLong *et al.* 1985; Lambrechts *et al.* 1989; Chadwick *et al.* 1997) or available commercially (Rodriguez *et al.* 2012b; Ahmed *et al.* 2015). The software minimises root mean squared differences between selected data points on the scan. Different forms of analysing these data to date have included maximum point loss (Lambrechts *et al.* 1989), mean profilometric loss (Pintado *et al.* 1997; Rodriguez *et al.* 2012a), volumetric loss (Pintado *et al.* 1997; Tantbirojn *et al.* 2012) and percentage of surface area affected (Chadwick *et al.* 2005).

Lambrechts *et al.* 1984 was the first to attempt to use surface matching software to detect differences between digitally aligned scans. A study was designed to measure attritional wear on the occlusal contact surface of molars and premolars (Lambrechts *et al.* 1984). Impressions were taken of volunteers (n=21) and repeated after 4 years. Impressions were electroplated with copper and poured in a gypsum cast. Using a depth measuring electrical probe, the profile of the surface was established. Data were superimposed using a computer algorithm based upon minimising the root mean squared distance between digitised surfaces. The process accuracy was reported as 1 μm , which was estimated by assessing the

deviation between four impressions made of a cast aluminium tooth. After 4 years the mean maximum point loss of profilometric depth in the centre of the enamel occlusal contact area was reported as 153 μm on molars and 88 μm on premolars. The authors reported that this equated to 11 μm on premolars and 21 μm on molars after 6 months. Although this method pioneered future research in this area, the electroplating method has been criticised as being time consuming and needing specialised equipment (Chadwick *et al.* 2002). The reliance on the accuracy of a single profilometric measurement to record all wear over a 4 year period may have increased the error. They also had not tested their measurement error in a clinical environment. Pintado *et al.* 1997 developed this technique further, utilising their self-developed software to investigate volume loss and mean profilometric loss over a 2 year period (Pintado *et al.* 1997). Following scavenger impressions, polyvinyl siloxane impressions were taken at baseline and repeated at 2 years. Impressions were cast using epoxy resin and scanned using a contact stylus profilometer with a 300 μm diameter and collecting data points every 50 μm (termed a 50 μm stopover). Using surface matching software, root mean squared differences were minimised between aligned digital images. The increase in wear facets on occlusal areas, volume loss and mean depth loss in microns were analysed. Mean volume loss per tooth was 0.04 mm^3 and mean depth loss was reported to be 10.7 μm per year. The authors noted that these were average values and individual cases can far exceed this wear rate. Although you can observe areas of volume gain in the images provided in the text, this error was not reported in the text.

From 1997 to 2005, Chadwick *et al.* developed and validated their system to measure wear progression on teeth and dental materials (Chadwick *et al.* 1997; Chadwick *et al.* 2002; Mitchell *et al.* 2003; Chadwick *et al.* 2004; Chadwick *et al.* 2005). Silicone impressions were taken at separate time intervals, coated in a high silver content electrical paint and a gel applied which was allowed to chemically harden. The impression was then poured in gypsum. This resulted in an electro-conductive layer similar to Lambrechts *et al.* 1984, which was digitised using an electrical probe (diameter 125 μm). Data were superimposed, again minimising root mean squared values, using purpose built superimposition software (SMADDA: surface matching and difference detection algorithm). This generated a colour imaged profile of the tooth (Figure 9).

Figure 9: Image of SMADDA colour mapping of loss. Yellow indicates insignificant wear, red indicates wear 100-200 μm , green indicates wear of 201-300 μm and blue indicates wear of 301-400 μm



The authors identified several problems with this method of analysis. The inherent problem with best-fit analysis is that it automatically attempts to eliminate differences between the two scans. Furthermore, as measurements are not taken from the same surface points, linear interpolation (method of fitting to construct

new data) is unavoidable (Mitchell *et al.* 2004). Any wide point separation will influence the overall fit chosen by the software. They attempted to overcome this by eliminating areas with loss greater than 50 μm in a refined best-fit analysis, which was then aligned using areas that represented little or no change. They also observed that reporting the percentage of surface loss over 50 μm improved accuracy of their data and chose to report the percentage of the surface affected by loss rather than mean profilometric depth. In their clinical trial analysing the palatal surfaces of the central incisors of 251 schoolchildren aged 11-13 over an 18-month period, 38 out of 265 surfaces displayed tooth surface wear. The majority of surfaces (n=21) demonstrated loss <5% of the tooth surface (Chadwick *et al.* 2005).

Rodriguez *et al.* 2012 used commercial engineering software, Geomagic Qualify, to perform a similar surface matching best-fit algorithm. Subjects with erosive tooth wear (n=63) had impressions taken at baseline and 6 months later. In addition, thirty subjects were followed up at a 12-month interval. Their casting methodologies were different as Rodriguez poured up impressions in type 4 stone directly. Despite this, both groups reported similar measurement error techniques for the process of $\pm 15 \mu\text{m}$. This was assessed via each researcher taking 5 impressions of either a volunteer (Rodriguez *et al.*) or a phantom head tooth (Chadwick *et al.*), casting them and analysing differences between samples. Rodriguez *et al.* chose median profilometric loss as their method of data analysis and reported that the majority of participants demonstrated wear less than 15 μm over a 6-month period with the largest median profilometric wear at patient level being 34.6 μm (IQR: 21.3-41.4).

There are several improvements in Rodriguez's method. The software and materials used are commercially available and can be readily utilised by other research groups. The smaller spot size of the laser (30 μm compared to stylus diameter 125 μm) would also allow for capture of more surface detail and the use of a non-contacting laser can more accurately reduce scanning time (for full discussion of profilometers see section 1.5.5.2). As a result of the time intensive scanning, Chadwick adopted a stopover of 150 μm (data point measurement every 150 μm intervals) compared to the 50 μm stopover adopted by both Rodriguez *et al.* and Pintado *et al.* The additional error of this decreased detail was estimated to be 26 μm compared to a 3 μm error rate when a 50 μm stopover rate was used (Mitchell *et al.* 2004; Rodriguez *et al.* 2012a).

However, there were also disadvantages in the Rodriguez *et al.* method. Type 4 dental stone is porous and when scanned by a laser with a small spot size it may result in overestimation of the wear, despite the lengths taken by the authors to minimise deviations in the casting technique. Furthermore, in their analysis they reported mean profilometric loss values, stating that profilometric gain was impossible, and so eliminated positive values. This method ignores the true extent of interpolation errors and may underestimate or overestimate true loss.

The most recent study to date using surface matching software utilised a CAD-CAM optical scanner as a method to digitise impressions (Tantbirojn *et al.* 2012).

Impressions were taken of participants with gastro-oesophageal reflux disease (n=12) and controls (n=6) at baseline and again six months later. The scans were digitised and superimposed using the same software as Pintado *et al.* 1997. They manually selected areas to perform volumetric analysis on and included only data

points showing observations greater than 20 μm (their estimated measurement error). The authors observed the total full arch volumetric loss to be 1.78 (SD 1.49) mm^3 for reflux patients and 0.42 (SD 0.27) mm^3 for controls. Although the sample size is small and the standard deviations are large they noted statistical differences between the different wear types of attrition, abrasion and erosion. The authors again noted that there was wide individual variation between participants with large outliers.

There are inherent errors in the use of superimposition and surface matching software. The aim of the superimposition process is to find the spatial relationship between scans which brings the surfaces into closest contact. Therefore, a best fit analysis will always attempt to minimise the deviation from identified points on the surface. This potentially minimises the profilometric surface loss. Furthermore, as many data points are collected, localised areas of severe loss may be under-reported when these values are averaged over the whole tooth surface.

Chadwick *et al.* 2004 attempted to bypass these faults by combining quantitative data and colour coded surface representation plots and used the following index which allowed general dental practitioners to quantify tooth wear (Chadwick *et al.* 2004).

1. Majority of surface unchanged with 5% or less exhibiting tooth surface loss
2. Majority of surface unchanged with 6–15% exhibiting tooth surface loss
3. Majority of surface unchanged with 16–25% exhibiting tooth surface loss
4. 26–50% of the surface exhibits tooth surface loss
5. 51% or greater of the surface exhibits tooth surface loss

This method has not been utilised elsewhere to date. A satisfactory method of overcoming inherent errors introduced via the best fit analysis has yet to be reported.

1.4.3.3 Other quantitative in vivo measurement methods

Other methods of quantitatively measuring erosive tooth wear in vivo have been attempted. Young *et al.* investigated the immediate protective effect of dentifrices in vivo by performing calcium analysis on collected citric acid which had been dispensed on isolated anterior teeth (Young *et al.* 2006). Although quick and inexpensive, it is difficult to ensure the same section of the tooth is used at each stage and the acid is effectively collected. Another research group have been developing an optical handheld device (Rakhmatullina *et al.* 2011; Carvalho *et al.* 2016) which claims that specular reflectance can assess erosion in vivo. Both of these methods provide a single snapshot of the stage of erosion and require further exploratory work before being used in vivo. Huysmans and Thijssen 2000 proposed the use of ultrasound to measure enamel thickness from the dentine enamel junction as a method to detect wear (Huysmans and Thijssen 2000). This was validated in vitro using extracted teeth but there have been no subsequent follow-up clinical trials published to date. Colour measurements have also been attempted by the same group however the individual colour variation was found to be too great a confounding factor to make it viable (Krikken *et al.* 2008).

Quantitative measurement of radiographs to measure physiological wear on incisor crown height was employed by Ray *et al.* 2015 (Ray *et al.* 2015). However, there is an ethical quandary over the justification of radiographic exposure to measure symptom-free teeth, in addition to difficulties reproducing the angle at

which the radiograph was taken at each visit. An accepted, easily employable method to accurately measure tooth wear in vivo has yet to be established.

1.4.4 THE USE OF INDEX TEETH TO MEASURE EROSIVE TOOTH WEAR

Epidemiologically, erosive tooth wear most commonly affects the maxillary incisor teeth and the occlusal surfaces of the lower first molars (Jaeggi and Lussi 2014).

When assessing erosive wear epidemiologically, some authors have assessed all surfaces (Lussi *et al.* 1991; Larsen *et al.* 2000; El Aidi *et al.* 2010) whereas others have based the assessment on first molars and incisors (Nunn *et al.* 2003; Bardsley *et al.* 2004; Dugmore and Rock 2004b). Others have reported the worst surface affected per tooth to grade the wear (Millward *et al.* 1994). Recently, a study compared whole mouth wear assessment with the use of index teeth and found no statistical differences between measurements, although the use of index teeth produced slightly lower values for wear (Al-Ashtal *et al.* 2016).

When performing quantitative wear analysis, analysing each tooth poses time constraints. Authors have recorded, scanned and analysed whole mouth wear (Lambrechts *et al.* 1989; Pintado *et al.* 1997; Rodriguez *et al.* 2012a). Others have focused upon reference teeth stating advantages of increased accuracy and repeatability in addition to reducing the scanning and data analysis time (Chadwick *et al.* 2005; Sundaram *et al.* 2007). No studies have compared whole mouth scanning and superimposition data with scanning data from index teeth although Rodriguez *et al.* recommended that index teeth be used in future studies (Rodriguez *et al.* 2012a).

1.4.5 *IN VITRO ASSESSMENT OF EROSIVE TOOTH WEAR*

Erosive tooth wear is an evolving process involving stages of demineralisation or softening of the dental surface and irreversible profilometric tissue loss. Several reviews conducted on the in vitro measurement of tooth wear have found no one technique to be suitable for measuring all stages of erosion (Barbour and Rees 2004; Field *et al.* 2010; Schlueter *et al.* 2011). This literature review will provide a brief overview of some of the more commonly used laboratory techniques to quantify demineralisation/softening and profilometric tissue loss. Surface microhardness testing and non-contacting laser profilometry are the methods of analysis employed within this thesis and shall be reviewed in greater detail.

1.4.5.1 Methods of measuring surface demineralisation

1.4.5.1.1 Microhardness and nanoindentation

The early stage of enamel erosion is the initial softening of the surface and subsurface following ion dissolution and mineral loss. This demineralisation results in surface hardness change and has been recorded in situ after as little as 90 seconds of acid exposure (Attin *et al.* 2001). The process of hardness testing involves indenting the surface with a variable force and loading time and analysing the length of the indentation to determine hardness (Attin *et al.* 1997). Knoop and Vickers diamonds are the most common forms of indents (Field *et al.* 2010). The Vickers diamond penetrates the surface to a greater extent (Shellis *et al.* 2011). Readings have been shown to be affected by the surrounding material and the intact underlying surface and thus are not as widely used in the literature (Shellis *et al.* 2011). For similar reasons, smaller indents made by nanoindentation (typically 200 nm) are more accurate and sensitive than microindentation (typically tens of μm). Nanoindentation is also able to measure the elastic modulus of the surface as tip displacement as a function of the load is constantly being

measured. This has been shown to be important in the detection of an intact subsurface area (Barbour and Rees 2004). A smooth, planar surface is required for hardness testing necessitating destructive sample preparation. This is of concern when measuring enamel as hardness has been observed to decrease with distance from the enamel surface at a rate of -0.23 KHN (Knoop Hardness Number) per μm (Meredith *et al.* 1996). It also limits the ability to compare hardness change to the original baseline as enamel layers underneath will be marginally softer regardless of the level of erosion (Carvalho and Lussi 2015). For this reason authors have recommended that hardness pre-testing should be performed to ensure that the hardness falls within a certain baseline hardness range (Lussi *et al.* 2011). Meredith *et al.* reported hardness values for enamel to range from 272 to 440 KHN (Meredith *et al.* 1996). This was also observed by Lussi *et al.* 2011 whereby applying a load of 100g resulted in ranges of 280-390 KHN (Lussi *et al.* 2011).

Furthermore, the relationship between surface hardness and erosion may not be linear. As dissolution proceeds, Barbour *et al.* 2003 suggested there is a minimum hardness value of softened enamel which will plateau at a certain level of erosive challenge (Barbour *et al.* 2003). This plateau was also observed in a 5 day in vitro cycling model by Venasakulchai *et al.* 2010 (Venasakulchai *et al.* 2010). Hara and Zero 2008 were unable to obtain a conclusive microhardness reading after 30 minutes of erosive challenge (Hara and Zero 2008). They concluded that microhardness change is suitable for measuring surface softening but may not be suitable for measuring erosion when profilometric tissue loss has occurred.

1.4.5.1.2 Calcium and phosphate mineral loss

Calcium and phosphate mineral analysis involves collecting the fluid/acid surrounding the enamel and analysing the mineral content at baseline and after an erosive challenge. Additional calcium or phosphate minerals present after the erosive challenge are hypothesised to be extracted from the hydroxyapatite structure. The advantages of this technique is that it can be used at a very early stage of erosion. Willershausen *et al.* 2009 detected quantifiable mineral loss after five seconds of erosion in vitro and has also been used in vivo (Young *et al.* 2006). The disadvantages of this technique is that it is an indirect measurement of erosion and re-precipitation of the minerals can occur (Shellis *et al.* 2011).

1.4.5.1.3 Quantitative laser fluorescence (QLF)

QLF uses a light or laser to cause fluorescence within the enamel. The change in intensity is measured on the tooth surface which can be correlated with demineralisation (Nakata *et al.* 2009). This can report lesion size, subsurface mineral loss and volume but cannot measure bulk tooth surface loss (Chew *et al.* 2014).

1.4.5.2 Surface profilometry

1.4.5.2.1 Surface profilometry overview

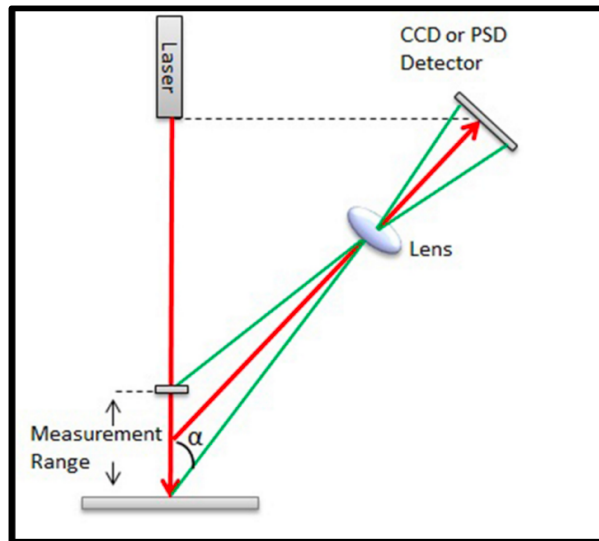
Surface profilometry uses differences in the texture and height between a reference area and a test area to accurately generate a profilometric measurement of erosive tooth wear (Heurich *et al.* 2010; Paepegaey *et al.* 2013). The two types of profilometry are contacting, via the use of a stylus probe or non-contacting, via the use of a laser/light.

All profilometers consist of a detector, determining where the data points on the sample are to be collected and the sample stage to hold the sample. Contacting

profilometry physically moves a stylus (typical diameter ranging 2-100 μm) along the surface in order to acquire the surface height. A feedback loop monitors the resistance from the sample pushing up against the probe to establish a surface profile. As it requires physical movements it can be slower than optical profilometry. The stylus tip can become contaminated by the surface and damage to the sample has also been reported. Non-contacting surface profilometry uses the reflectance of lasers to establish an accurate profile, directing the laser in order to obtain a three dimensional reading of the surface. Non-contacting laser scans can however be affected by environmental factors such as vibrations/temperature within the laboratory and the angle, colour and transparency of the surface, (Hewlett *et al.* 1992; Rodriguez *et al.* 2009).

The width of the measuring tip, affects the accuracy of both types of profilometer. A wider stylus tip or laser spot size will not penetrate troughs present on the surface. However, the increased amount of data captured by a smaller tip or spot size may create noise within the data (Field *et al.* 2010). Different sizes of probes or lasers can be utilised depending on the topography of the surface to be measured and the level of detail required. The spot size of a laser, equivalent of stylus diameter size, typically ranges from 2-50 μm . The smaller the spot size the greater amount of detail that can be detected. This also decreases the measurement range as shown in figure 10.

Figure 10: Determination of measurement range in profilometry



The number of readings taken per surface area also affects the accuracy of data with increasing data points reducing the measurement error but increasing the time necessary for data collection (Mitchell *et al.* 2003; Rodriguez *et al.* 2012b). Rodriguez *et al.* 2012 observed that when readings were taken every 15 μm , 50 μm , 75 μm and 100 μm , respective errors when measuring the same accurate slip gauge increased from 2 μm to 2.6 μm to 3 μm to 3.6 μm respectively (Rodriguez *et al.* 2012b).

Profilometric measurements in enamel typically measure change in height of an exposed enamel surface against an unexposed or unaffected reference area. Step height can be analysed using different methods. Older studies used one measurement reading from a single step height profile (Attin *et al.* 2009), often take at the mid-point. This carries a risk of the data not being representative of the entire sample and it has been recommended to take an average of several readings (Rodriguez and Bartlett 2010) which is the method employed within the in vitro

work of this thesis. Unpublished work by our group (Mistry 2016) observed that although there were no statistical differences between profile measurements obtained from a single reading and an average of multiple readings, the standard deviations were much lower within groups when multiple readings were obtained.

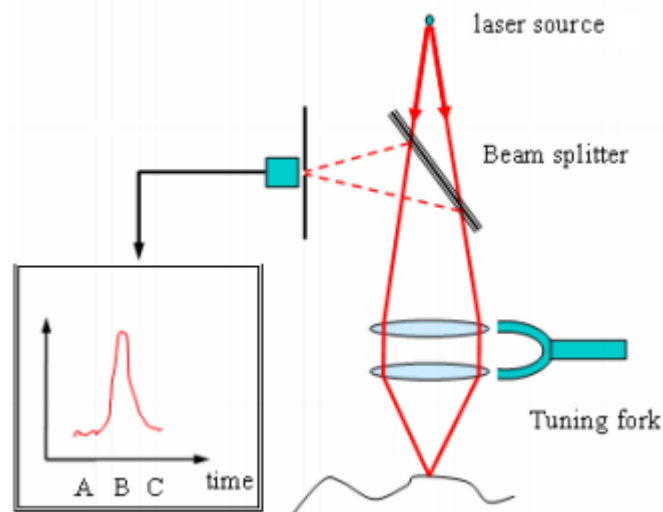
If it is feasible to scan the entire surface, a full 3D representation of the surface can be produced. When this method is employed, imaging software can also be utilised to calculate volume changes (Paepegaey *et al.* 2013) making it a better reflection of the entire sample. Analysing volume change in addition to profilometric loss also increases the information available to the researcher. However, volume measurements will be affected if there are undetected cracks or defects in the enamel surface which could normally be avoided when multiple profile measurements are being analysed. Scanning the entire surface also takes more time.

A disadvantage of profilometry is that bulk surface loss needs to occur before erosion can be detected by the profilometer. This makes profilometry unsuitable for detection of early stages of erosion (Hara and Zero 2008). To measure step height at a micron level, surfaces also need to be flat in order to generate an accurate step height profile. The polishing process is only accurate to within $\pm 1 \mu\text{m}$ which may affect overall accuracy (Austin 2011). Despite this, profilometry remains an accurate and suitable method for measuring profilometric dental tissue loss in vitro with measurements between different profilometers highly correlated (Paepegaey *et al.* 2013).

1.4.5.2.2 Different types of laser profilometers

The accuracy of the laser profilometer depends on the spot size and the resolution of the laser. There are two main types of lasers, confocal lasers and triangulation lasers. Both are used within this thesis depending on the surface to be measured. Confocal lasers typically have a small spot size (2-10 μm), high vertical resolution ($\sim 1\text{ nm}$) and a narrow depth of focus. Confocal lasers direct the beam in a concise method through a lens that vibrates vertically at high speed through the use of a tuning fork. As the light is reflected off the target surface it converges before entering the light receiving element. The height is measured by determining the exact position of the lens as the light hits the receiving element. These precise measurements are ideal for capturing increased detail with a small vertical depth of focus. A schematic can be observed in Figure 11.

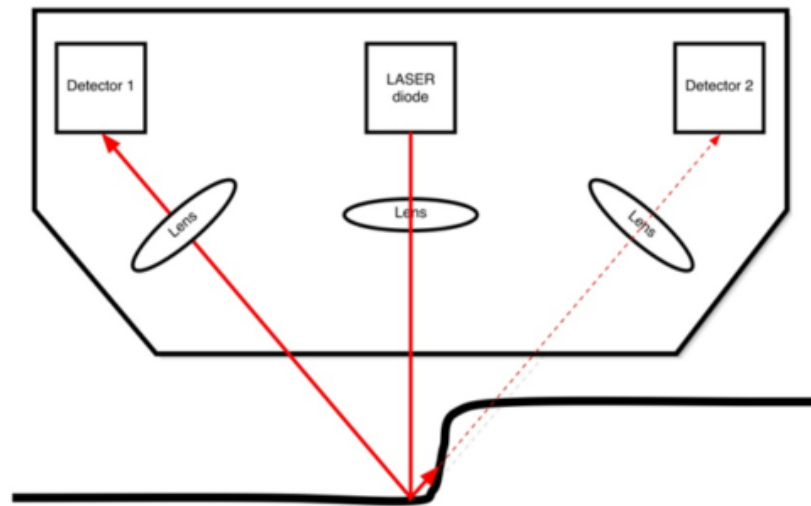
Figure 11: Schematic of confocal laser profilometer



In contrast, triangulation lasers have a larger spot size (typically 20-50 μm), a sensor resolution of 0.1 μm and a larger depth of focus. This makes them useful when measuring larger heights and macro topography of an entire dental surface. Triangulation lasers scatter light on the surface through a lens. A further two lens

are set up to catch the reflected light and focus the light onto the detector. Changes in the topography of the surface cause displacement of light on the detector, which uses the level of displacement to measure the topographical changes. A schematic can be viewed in Figure 12.

Figure 12: Schematic of triangulation laser profilometer (Austin 2011)



1.4.5.2.3 Comparison of the lasers and possible errors

Both laser profilometers have inherent errors. Sharp edges may cause the laser stylus to overshoot at the bottom of grooves resulting in artificial increased step height (Whitehead *et al.* 1999). This is particularly true for the confocal laser which has one signal detector. If a sharp edge interferes with the light returning to the detector this will result in overshooting or data drop-out. The second signal detector in the triangulation laser overcomes this issue as is shown in the schematic in Figure 12. The confocal laser has increased resolution and accuracy. However, the disadvantages are the reduced Z height within which the laser can measure and the increased time necessary for scanning. Conversely the triangulation laser allows more freedom with variations in the Z height of the surface to be measured with a reduction in the resolution of the laser. A further

disadvantage of the triangulation laser is that reflective, shiny surfaces are difficult to measure as the mechanism of action relies on scattering of light. If the light reflects directly back this will cause error within the data (Rodriguez *et al.* 2009).

Although it is important to ensure laboratory conditions are as similar as possible when carrying out measurements, due to its increased accuracy, the confocal laser will be more susceptible to changes environmental factors. This is particularly true for changes in temperature which can affect the degree of vibration of the tuning fork (Whitehead *et al.* 1999).

1.4.5.3 Other quantitative methods of interest

1.4.5.3.1 Atomic force microscopy (AFM)

Atomic force microscopy is a high-resolution form of microscopy which uses minute forces to scan the specimen surface to give a very detailed picture of the surface topography. It can quantify bulk surface loss in addition to accurately displaying the surface topography. AFM has a limited scan size which can take long periods of time. The force contact can also result in damage to a fragile surface (Field *et al.* 2010).

1.4.5.3.2 Microradiography

Low energy x-rays penetrate the sample which can then produce an enlarged image of the surface. This allows detection of bulk surface loss and can show subsurface demineralisation using microdensity software.

1.4.5.3.3 *Optical coherence tomography (OCT)*

Optical Coherence Tomography uses light in a similar manner to ultrasound imaging to form a cross sectional image. It uses the magnitude and echo time delay of light to produce a qualitative image of the surface structures. In addition, the depth of the lesion can be analysed quantitatively. This method has been attempted to quantify tooth wear in vivo, although reports from preliminary studies have observed that there was not enough change within the enamel to accurately measure early erosion (Chew *et al.* 2014).

1.4.5.4 **Methods to measure surface topography**

1.4.5.4.1 *Surface roughness*

Surface roughness may be described as deviation from the form of a surface.

Multiple parameters have been used to measure this deviation from the form. Field *et al.* 2010 observed the most common parameter for erosion studies to be the two-dimensional arithmetic average of the vertical deviation from the form (Ra) and its three-dimensional counterpart (Sa). Two other forms of measurement commonly used are area scale analysis and the bearing curve. Area scale analysis uses a combination of height and spacing in analysis software to determine the form over an area (Hyde *et al.* 2014; Leach 2014). The bearing curve is a calculation of amplitude and spacing to get one overall profile for the roughness of the sample (Field *et al.* 2010; Leach 2014). A recent paper by Hara *et al.* reported that different surface textures could differentiate between different erosive tooth wear mechanisms (Hara *et al.* 2016) There is also potential for surface roughness measurements to be used in vivo (Carvalho *et al.* 2016).

1.4.5.4.2 *Scanning electron microscopy*

Scanning electron microscopy (SEM) produces high-resolution 3D images by coating the surface of the sample in an electronically conductive material and

bombarding it with electrons. Analysis of the reflection produces a high-resolution qualitative image but is unable to give any quantitative data. The sample is also destroyed in the process. This is useful to give a greater understanding of what is happening on the dental surface when used in conjunction with quantitative data.

1.4.5.5 Preparation of samples for in vitro enamel investigations

There are several variables which may impact upon the results of in vitro enamel investigations (Mistry *et al.* 2015). Although there is an increasing trend to use alternative methods of measurement on natural, minimally prepared enamel (Hara *et al.* 2016), conventional methods of erosive wear assessment often require enamel samples to be modified and polished (Field *et al.* 2010). This typically involves sectioning an enamel surface from a caries-free tooth, mounting and performing a polishing regime to bring it within a certain flatness tolerance. This preparation is performed to maximise the accuracy of the measuring systems, most commonly profilometers and microhardness testing.

Human enamel is more clinically relevant although bovine enamel is also often used (Wegehaupt *et al.* 2008). One in vitro study observed that profilometric loss progressed 30% faster when bovine enamel was used (White *et al.* 2010).

Furthermore the type of tooth (molar or premolar) has been observed to have differing levels of susceptibility to microhardness change although no differences were observed with profilometry data (Carvalho and Lussi 2015; Mistry *et al.* 2015). The surface of the enamel to be tested also requires consideration. Some studies have observed differences in microhardness measurements between erosive wear measurements made on buccal and lingual surfaces subjected to the same erosive regime (Mistry *et al.* 2015) and others have not (Carvalho and Lussi 2015). Once the enamel has been mounted it needs to be subjected to a polishing

regime. The more abrasive the polishing regime, the more surface enamel is removed and the surface becomes softer as measured by hardness testing (Meredith *et al.* 1996). Carvalho *et al.* recently observed that the susceptibility of enamel to initial erosion in vitro was dependent on depth from the surface for microhardness and calcium release methods (Carvalho and Lussi 2015). Polishing leaves a smear layer of debris occluding the enamel prisms. Mistry *et al.* observed that not removing this smear layer offered a protective effect in vitro (Mistry *et al.* 2015). The method of sample storage, in water, 100% humidity or dry, has been observed to impact significantly on dentine samples (Attin *et al.* 2009) but not enamel samples (Attin *et al.* 2009; Mistry *et al.* 2015). The use of saliva in in vitro models is outside the scope of this thesis, however the use of artificial saliva or natural saliva also significantly impacts the level of erosive wear observed (Baumann *et al.* 2016).

The severity of the erosive challenge depends on the research hypothesis and the method of measurement (Stenhagen *et al.* 2010). One can vary the type, concentration, pH and the immersion time of the acid. As this thesis focuses on dietary erosive wear, citric acid, the most common form of dietary acid at a concentration and pH commonly found in dietary acids was chosen. Increasing immersion time in an acidic solution will increase the erosive wear observed (Stenhagen *et al.* 2010).

Although each variable chosen will impact the results, standardising each step and ensuring good scientific protocol is followed will ensure comparisons within the study are valid (Mistry *et al.* 2015).

1.5 SUMMARY AND AIM OF RESEARCH

Although the field of erosive wear is being continually researched, there are no evidence-based guidelines regarding the timing of dietary acid intake, oral hygiene procedures and erosive tooth wear. There is ambiguity regarding the optimal timing of fluoride application in relation to an acidic challenge in vitro. No clinical studies to date contrasted brushing immediately after a meal and brushing immediately after consuming a dietary acid. There are also no clinical studies contrasting the relative risk of consuming dietary acids with meals and between meals.

Furthermore, following identification of risk factors, it is unknown if providing advice will reduce the rate of erosive tooth wear progression. No interventional studies have been performed investigating if dietary advice, delivered within a dental setting, can be effective at delaying tooth wear progression.

The overall aim of this research was to investigate the role of timing of oral hygiene procedures and dietary acid intake in erosive tooth wear progression.

This investigation occurred in four parts.

1. A laboratory investigation into the timing of sodium fluoride and stannous fluoride application before or after an erosive challenge. This study also served as a training tool in laboratory experiments and analysis.
2. Development and validation of a questionnaire to assess timing of dietary acid intake and tooth brushing.
3. A case-control investigation study on 600 participants investigating the associations of dietary acid intake and tooth brushing with severe erosive tooth wear.

4. A randomised controlled clinical trial investigating the effectiveness of two forms of dietary advice as an intervention on tooth wear progression.

The null hypotheses proposed for this study are

1. There will be no difference in erosion comparing stannous and sodium fluoride on enamel samples applied before or after an acidic challenge in vitro.
2. There will be no association between the frequency of dietary acid intake and severe erosive tooth wear.
3. There will be no association between erosive tooth wear and the duration of consumption of dietary acids.
4. There will be no association between severe erosive tooth wear and the timing of tooth brushing to meals or dietary acid consumption.
5. A behaviour change intervention will not change dietary acid intake compared to standard of care dietary advice.
6. A behaviour change intervention will not impact tooth brushing behaviours compared to standard of care dietary advice.
7. A behaviour change intervention will not change the progression of erosive tooth wear.

CHAPTER 2: OPTIMAL TIMING OF STANNOUS AND SODIUM FLUORIDE MOUTHRINSE IN RELATION TO AN EROSIVE CHALLENGE

2.1 OVERVIEW

The effectiveness of fluoride in protection against erosive tooth wear in the literature is controversial and may be dependent on the frequency of application (Hystad Hove *et al.* 2014), type (Stenhagen *et al.* 2013), concentration (Austin *et al.* 2010) and pH (Attin *et al.* 1999) of the fluoride preparation. This laboratory study investigated if the timing of the fluoride application, in relation to an erosive challenge, had an impact on the level of protection conferred by the fluoride preparation. A fluoride-containing mouth rinse applied after an erosive challenge may offer the remineralisation benefits of fluoride without the concomitant mechanical insult of tooth brushing. Conversely, fluoride application before an erosive challenge may offer an improved level of surface protection. This may result in a reduction in the overall wear of the enamel surface. Sodium fluoride is widely available commercially and has been shown to result in less erosion when applied both before (Hughes *et al.* 2004) and after (Ganss *et al.* 2008; Mathews *et al.* 2012) a dietary acidic challenge. Stannous fluoride has shown very promising results in dental erosion studies where it has been applied before (Faller *et al.* 2014) and after dental erosion (Hove *et al.* 2008).

The aim of this study was to perform a laboratory investigation into the optimal timing of sodium fluoride and stannous fluoride; either before or after an erosive challenge. This study also served as a training tool in laboratory experiments and analysis.

2.2 OBJECTIVES

- To assess if stannous fluoride or sodium fluoride is optimally applied before or after an erosive challenge in vitro using step height and microhardness change measurements.
- To compare the level of protection conferred by stannous fluoride and sodium fluoride when applied either before or after and erosive challenge using step height and microhardness change measurements.
- To assess if the effect observed after one experiment cycle is replicated after five experiment cycles.

2.3 NULL HYPOTHESIS

The null hypothesis is that

1. There will be no difference in erosion comparing stannous and sodium fluoride on enamel samples applied before or after an acidic challenge in vitro.

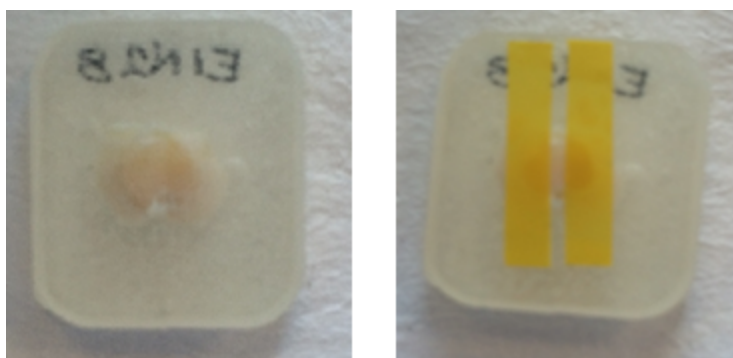
2.4 MATERIALS AND METHODS

2.4.1 ENAMEL SAMPLE PREPARATION

Previously extracted, caries-free human molars were collected (n=80, REC ref 12/LO/1836) and stored in sodium hypochlorite solution for a minimum of 3 days. The buccal surfaces were sectioned using a circular saw (Isomet 1000 with an Extex diamond wafering blade; Buehler, Coventry, UK) at a speed of 300 rpm and force of 150 g and placed into a custom-made silicone mould (specimen size 8 × 21.5 × 24 mm) and embedded in cold cure acrylic resin (Oracryl; Bracon, East Sussex, UK). Specimens were then polished (Metaserv 3000 variable speed

grinder-polisher; Buehler, Coventry, UK) using the Federation of European Producers of Abrasives (FEPA) standard silicon carbide sandpaper, starting at 80 grit, followed by the 180, 600, 1200, 2400 and 4000 grit. This resulted in a smooth, polished surface with a flatness tolerance $\pm 1\mu\text{m}$. Following polishing, specimens were immersed in 80 ml of deionised water and ultrasonicated (GP-70; Nusonics, Lakewood, US) at 60 Hz for 15 min, after which they were rinsed and allowed to air dry. Adhesive tape was placed on the enamel surface to create a window approximately 1 mm \times 3 mm wide with a reference area on either side. Specimens were stored in dry conditions prior to the erosive cycling.

Figure 13: Un-taped sample and final taped sample



2.4.2 SOLUTION PREPARATION

Acid and fluoride solutions were freshly made prior to use in the study.

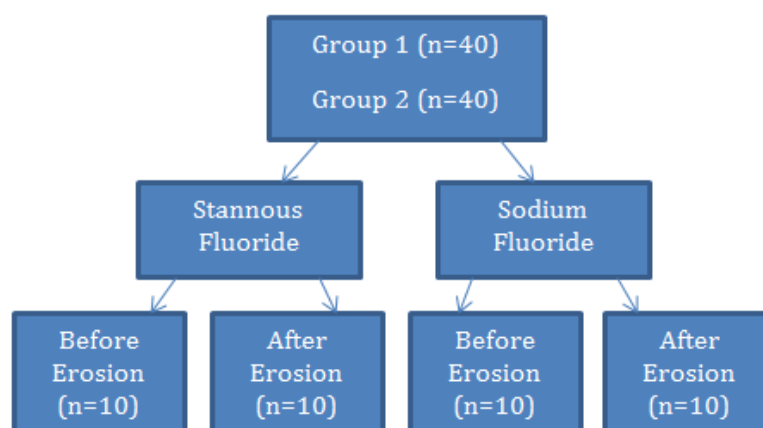
Commercially available sodium and stannous fluoride mouth rinses were used at a 225 ppm concentration. The sodium fluoride mouthrinse (Fluoriguard, alcohol free, sodium fluoride 0.05% w/w 225 ppm; Colgate, Surrey, UK) required no preparation and was used as supplied. The natural pH of this solution was pH 6.01 (SD= 0.04). The stannous fluoride mouthrinse (Periomed alcohol free, stannous fluoride 0.63% w/w, fluoride 0.12% w/w; 3M ESPE, Minnesota, US) was supplied

as concentrate and required diluting. To manufacture a solution containing 225 ppm fluoride, 93.75 ml of Periomed was diluted in 500 ml of deionised water and stirred for 5 minutes using a magnetic stirrer to ensure a uniform solution. The natural pH of this solution was pH 3.81 (SD = 0.09). The manufacturers of both products did not provide data on the level of available fluoride per ml in each mouthrinse. A 0.3% solution of citric acid was created by diluting 3 g of anhydrous citric acid powder (99%; Sigma Aldrich, Haverhill, UK) in 1000 ml of distilled water. The pH was adjusted to pH 3.2 (SD = 0.01) with 0.1 M sodium hydroxide (98%; Sigma Aldrich, Haverhill, UK).

2.4.3 EXPERIMENTAL PROCEDURE

Samples were randomly divided into two groups (n=40). Group 1 was subjected to one treatment cycle and group 2 subjected to five treatment cycles. Both groups tested 225 ppm stannous fluoride and sodium fluoride commercial mouth rinses. Within each group, samples (n=10) were randomly allocated to subgroups for each of stannous fluoride and sodium fluoride: fluoride applied before erosion and fluoride applied after the erosion.

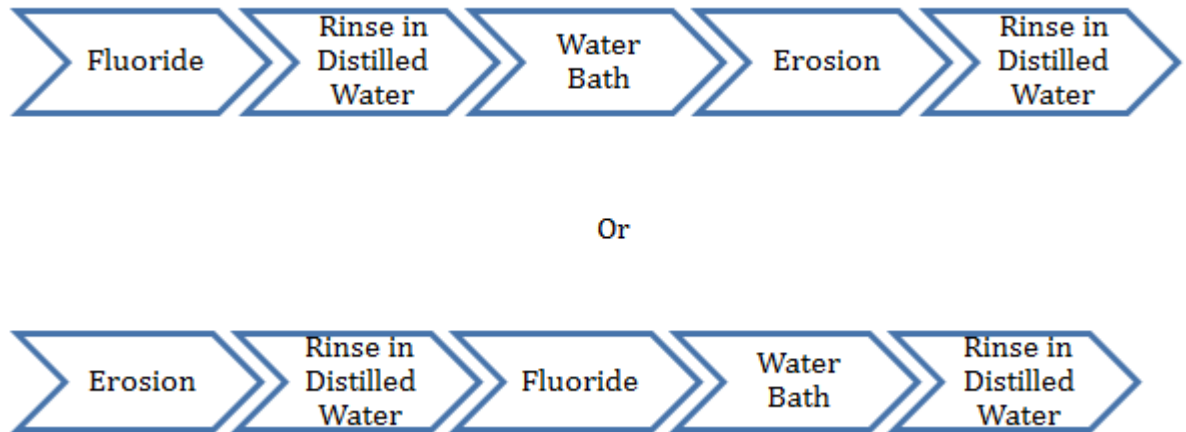
Figure 14: Random allocation of samples (n=80)



When the fluoride was to be applied before the erosive challenge, the specimens were immersed in 80 ml of either fluoride solution for 1 minute and agitated using an orbital shaker at 62.5 rpm (Stuart Orbital Shaker SS1; Bibby Scientific Limited, Staffordshire, UK). The specimens were rinsed for 2 minutes in distilled water using the orbital shaker at 62.5 rpm. This was followed by placement in a 100 ml distilled water bath, where samples were left, unstirred, for 30 minutes as per manufacturer's instructions. Following treatment, the samples were then immersed in 80 ml 0.3% citric acid, pH 3.2 for 10 minutes under agitation using the orbital shaker set at 62.5 rpm. Following the erosive challenge, each sample was rinsed in 100 ml distilled water for 2 minutes in the orbital shaker at 62.5 rpm. When the fluoride was to be applied after the erosive challenge, samples were immersed in 80 ml of 0.3% citric acid pH 3.2 for 10 minutes in the orbital shaker at 62.5 rpm. Samples were then rinsed in 100 ml of deionised water for 2 minutes in the orbital shaker set at 62.5 rpm. The fluoride was then applied by immersing samples in 80ml of fluoride solution for 1 minute in the orbital shaker set at 62.5 rpm. Samples were then rinsed again in 100 ml of deionised water for 2 minutes in

the orbital shaker before a wait period of 30 minutes in a 100 ml of distilled water bath.

Figure 15: Experiment flow chart

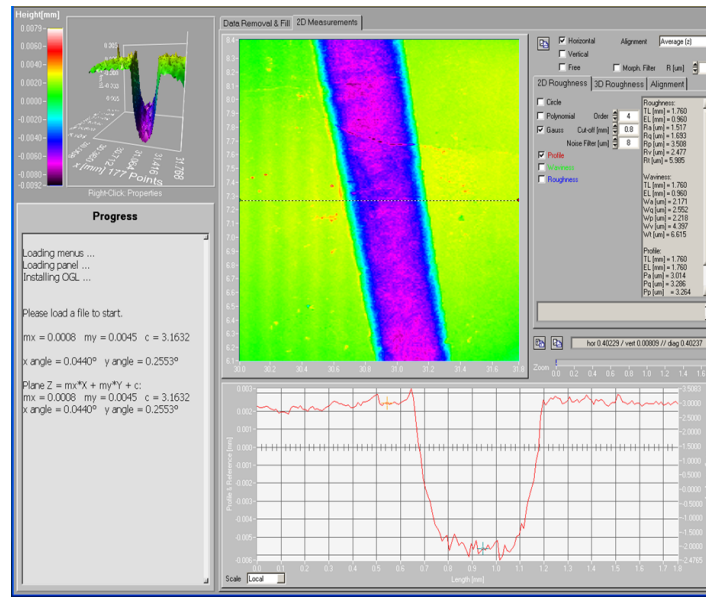


These cycles were repeated five times for group 2. The experiment was carried out at $20^{\circ}\text{C} \pm 1$. Samples were allowed to air-dry at room temperature for 12 hours and the tape was carefully removed.

2.4.4 TOOTH WEAR MEASUREMENTS

Removal of the tape resulted in two unaffected reference areas on either side of the affected eroded area. The step heights of the samples were measured using a confocal non-contacting red light laser profilometer (XYRIS 4000, Taicaan, UK) with a spot size of $2\text{ }\mu\text{m}$ and resolution of $0.01\text{ }\mu\text{m}$. A $2 \times 2\text{ mm}$ area of the sample was scanned ensuring equal widths of reference and eroded areas were captured. The sample was scanned in a raster pattern taking measurements every $10\text{ }\mu\text{m}$ in the X and Y direction. Data were analysed using Boddies v1.92 (Taicaan, UK) and can be observed in Figure 16. The yellow/green areas are the reference areas and the purple area in the centre is the eroded area.

Figure 16: Analysis of step height caused by an erosive challenge in vitro using BODDIES software



The step height was calculated by measuring the depth of the erosive wear from the reference area to the midpoint of the trough. Ten measurements were made per sample and the average of these readings calculated to establish the mean step height in microns (μm).

Surface microhardness was measured using a Knoop hardness tester (Duramin 2, Struers, Germany) using methods previously employed within our group. For each sample, three indentations (10 s dwell time with 981.2 mN loading) were made 100 μm apart in both the eroded area and the reference area. The mean of each of these were calculated and subtracted from each other to calculate the change in microhardness in Knoop Hardness Number (KHN).

2.4.5 STATISTICS

Statistical analysis was performed on IBM SPSS Statistics 22 (IBM Corporation, Armonk, New York). Data were assessed for normality using normality plots and Shapiro-Wilks tests. As the data were normally distributed, a three-way analysis of variance (ANOVA) was used to assess for group differences. Following this, post

hoc bonferroni was used to assess for individual differences. The level of significance was set at $P \leq 0.05$.

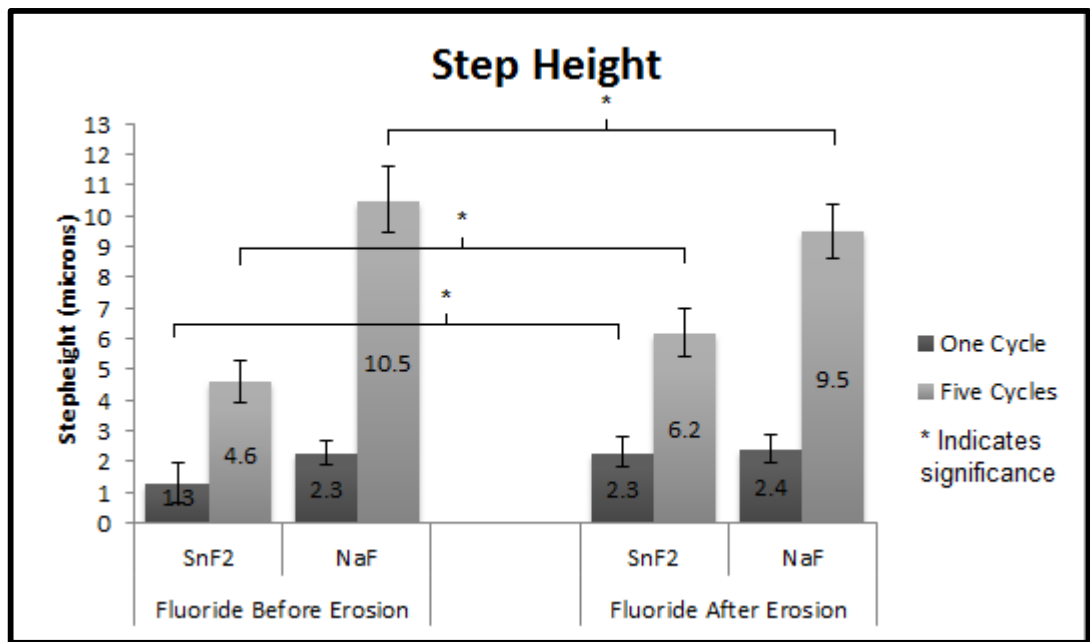
2.5 RESULTS

2.5.1 STEP HEIGHT RESULTS

The results for step height are displayed in Figure 17. For group 1 (one cycle) the mean step heights with standard deviations for stannous fluoride before and after erosion were $1.3 \mu\text{m}$ (0.63) and $2.3 \mu\text{m}$ (0.48) respectively. The mean step heights for sodium fluoride before and after were $2.3 \mu\text{m}$ (0.39) and $2.4 \mu\text{m}$ (0.46). Overall, application of stannous fluoride before erosion resulted in the least step height. This was statistically lower than stannous fluoride application after erosion ($p=0.001$) and sodium fluoride application both before and after erosion ($p<0.001$). No difference was observed in the step heights formed when comparing sodium fluoride application before and after erosion.

For group 2 (five cycles) the mean step heights with standard deviations for stannous fluoride before and after erosion were $3.2 \mu\text{m}$ (0.57) and $4.2 \mu\text{m}$ (0.7) respectively. The mean step heights for sodium fluoride before and after were $8.2 \mu\text{m}$ (0.65) and $7.5 \mu\text{m}$ (0.85). Stannous fluoride application resulted in less step height than sodium fluoride application, regardless of whether it was applied before or after erosion ($p<0.001$). Again, stannous fluoride applied before the erosive challenge resulted in significantly less step height than stannous fluoride applied after erosion ($p<0.001$). Interestingly, application of sodium fluoride after erosion resulted in statistically lower step height than application of sodium fluoride before erosion ($p=0.035$).

Figure 17: Step height results of fluoride application before or after erosion.

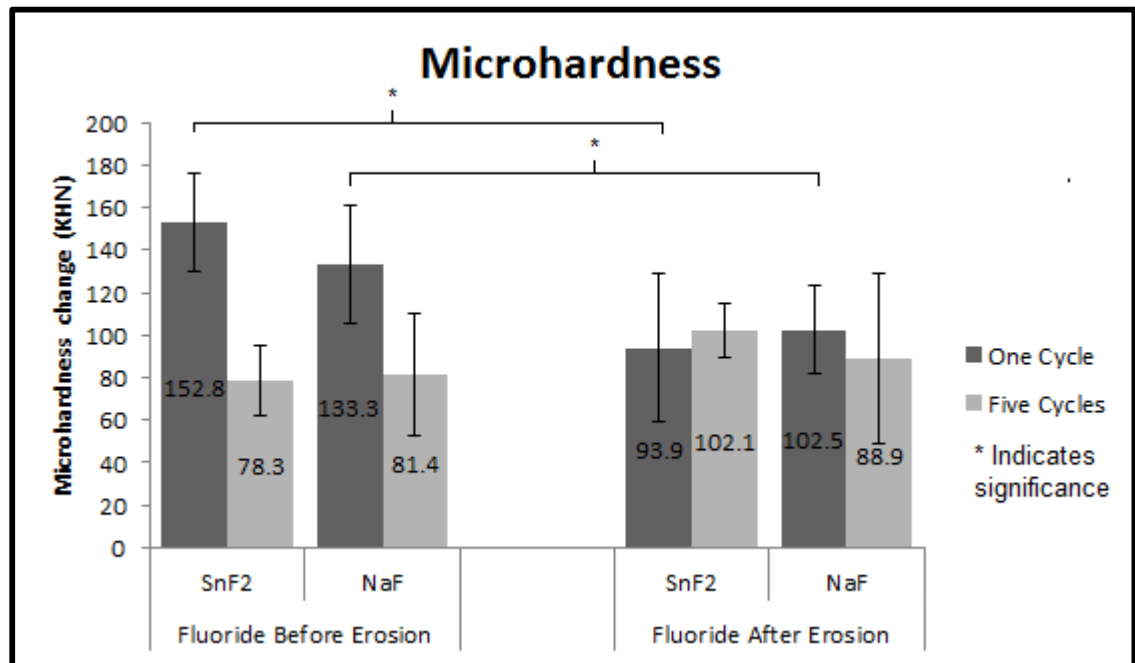


2.5.2 MICROHARDNESS RESULTS

For group 1 (one cycle) the mean microhardness changes with standard deviations for stannous fluoride before and after erosion were 152.8 KHN (22.9) and 93.9 KHN (34.7) respectively, and for sodium fluoride they were 133.3 KHN (27.6) and 102.1 KHN (21.1). Application of the fluoride before the erosive challenge resulted in a significantly increased microhardness change for both stannous fluoride ($p=0.011$) and sodium fluoride ($p=0.035$). However, there was no statistical difference between the fluorides. Overall, the microhardness change was greater for one cycle of erosion than for five cycles of erosion. For group 2 (five cycles) the mean microhardness changes (SD) for stannous fluoride before and after erosion were 78.3 KHN (16.6) and 102.1 KHN (12.6) respectively. The mean microhardness changes for sodium fluoride were 81.4 KHN (28.6) and 88.9 KHN (40.4). Application of stannous fluoride after the erosive challenge resulted in significantly increased microhardness change when compared to stannous fluoride

before the erosive challenge ($p=0.003$). There was no significant difference between the fluorides with respect to microhardness change.

Figure 18: Microhardness change results for fluoride before and after erosion.



2.6 DISCUSSION

In this laboratory investigation differences in step height and microhardness change were observed when the timing of fluoride application was altered with respect to the acid challenge. Furthermore, differences between the fluorides were observed, both in the level of protection conferred and in the optimal timing of application. Therefore, the null hypotheses were rejected.

Rinsing with stannous fluoride resulted in less step height than rinsing with sodium fluoride regardless of whether it was applied before or after erosion. This finding is consistent with the literature showing that stannous fluoride offers more protection than sodium fluoride in vitro (Wiegand, Bichsel, *et al.* 2009), in situ (Huysmans *et al.* 2011; Stenhagen *et al.* 2013) and in vivo (Young *et al.* 2006).

Only one study, to the author's knowledge, observed conflicting results when comparing the effectiveness of sodium and stannous fluoride (Barlow 2009). They observed that application of a sodium fluoride dentifrice after a 90 second in situ period resulted in a greater microhardness recovery compared to the stannous fluoride dentifrice. This assesses the remineralisation process rather than surface protection. In addition they measured microhardness only, and as observed in this study and others (Venasakulchai *et al.* 2010), it is not always an indicator of the level of wear present. The stannous fluoride dentifrice used in the study contained an anti-calculus agent which is known to cause mineral softening to prevent build-up of calculus which may have affected the result. Regardless, this study did show the remineralising potential of sodium fluoride, particularly when saliva is involved.

The novel finding in this study was that altering the timing of fluoride application produced statistical differences within groups and also the optimal timing of application was different for each fluoride. Stannous fluoride was optimally applied before the erosive challenge had occurred and this was true for both one cycle of erosion and five cycles of erosion. Conversely, sodium fluoride was optimally applied after the erosive challenge. This was only observed after five treatment cycles. This finding may not be surprising when considering the chemical properties of the different fluorides and how they react with the hydroxyapatite. Only one other study to the author's knowledge investigated the timing of fluoride application before and after an erosive challenge (Lussi *et al.* 2008). Lussi *et al.* applied four sodium fluoride toothpastes and one stannous fluoride containing toothpaste before and after a single erosive challenge. They

observed that there was less microhardness change when the toothpaste slurries were applied before the erosive challenge but did not observe any difference between the two fluorides. This confirms our fluoride microhardness data where a statistical difference in the timing of application was observed but no statistical difference between the fluorides was observed after one cycle of erosion. We did however observe statistical differences between the fluorides using our profilometric data. This may be due to the more aggressive erosion regime used in this study.

The remineralisation role of sodium fluoride in dental caries is well established (Marinho *et al.* 2009). Although an erosive lesion is different to the carious lesion there is evidence to show that remineralisation to a certain degree is possible (Shellis *et al.* 2014) and studies have shown that fluoride applied to an eroded lesion can result in increased hardening (Mathews *et al.* 2012). The higher pH of the sodium fluoride mouthrinse may also be an advantage, buffering the erosive challenge. Furthermore the presence of fluoride in an acidic environment will result in the incorporation of fluoroapatite in the dental surface (Shellis *et al.* 2014). As discussed in the literature review, this may make the enamel less prone to further mechanical and chemical degradation during future erosive challenges (Shellis *et al.* 2014) and may explain why there was no difference after one erosive cycle but a significant difference after five erosive cycles. Conversely, the surface protection effects of the fluoride ion in the literature are not consistent. Studies have applied sodium fluoride before an erosive challenge and found it to have a protective effect whereas others have found it similar to a water control (Willumsen *et al.* 2004; Hove *et al.* 2008). The ideal conditions for the prevention

of initial demineralisation for fluoride seem to be when the fluoride concentration is very high, when the fluoride is applied at a low pH and when the erosive challenge is relatively mild. There are studies that have applied sodium fluoride after erosion and found there to be no statistical difference to water/a negative control (Wiegand, Bichsel, *et al.* 2009; Hystad Hove *et al.* 2014). However, the majority of studies have observed a remineralisation effect with sodium fluoride, particularly with multiple applications and mild erosive challenges (Schlueter, Klimek, *et al.* 2009b; Ganss, Neutard, *et al.* 2010; Schlueter *et al.* 2010).

The chemistry of stannous fluoride has several inherent advantages making it more effective against an erosive challenge. It is chemically stable at a low pH of 3.5-4. This has been observed to react immediately with the enamel, incorporating minerals into the hydroxyapatite structure and forming stannous deposits on the surface (Schlueter, Hardt, *et al.* 2009). There are also two active components within stannous fluoride, the stannous (Sn^{2+}) ion and the fluoride (F^-) ion. Stannous alone has been observed to have a preventative effect against erosion when present in the form of stannous chloride (Ganss *et al.* 2008). The reactivity of stannous with the hydroxyapatite may free fluoride ions to play a role protecting against erosion. These preventive measures occur before an erosive challenge. Therefore, the enhanced protection observed with pre-treatment with stannous fluoride in this experiment might have been expected. For the same reasons, stannous fluoride applied after an erosive challenge would aid in remineralisation as both ions can be incorporated into hydroxyapatite. The surface is more resistant to a subsequent enamel challenge (Schlueter, Hardt, *et al.* 2009).

There are several improvements, which could be made to the methodology of this experiment. In this experiment, it is unclear due to the multiple applications of fluoride and acid immersion whether the result was remineralisation or surface protection. For example, the reduced step height observed when stannous fluoride was applied after an erosive challenge after five cycles of erosion could have been derived from the surface protection in the middle erosive cycles rather than the final remineralisation at the end of the five erosive cycles. However, the differences were statistically significant which implies that the sequence of fluoride application and erosion is important. A further limitation of this study is that distilled water was used during the wait period. In an oral environment the fluorides would be reacting with the salivary pellicle and intraoral saliva (Hara *et al.* 2013). Distilled water cannot replicate saliva and more in situ and in vivo studies are needed to establish the clinical relevance of this study's finding.

The materials used in this study were commercial mouth rinses. The manufacturers recommend that Periomed is diluted to 150ppm fluoride. However, a solution containing 225ppm was used in this study to compare equal amounts of the fluoride ion in order to establish the effects of the stannous ion in the solution. A wait time of 30 minutes was used to simulate the manufacturers request to not eat or drink anything after using the mouth rinse. Although this experiment was not designed to replicate the oral environment, this step was completed to complete the prescription of the mouth rinse.

Different commercial formulations have been observed to have different levels of fluoride availability despite similar fluoride concentrations (Hara *et al.* 2009). The level of fluoride availability for remineralisation/resistance of demineralisation

was not provided by the manufacturers was not assessed in this experiment.

Further evaluation of this is needed in addition to establishing the influence of saliva before any clinical implications can be made.

The vertical step height for this experiment was measured using non-contact laser profilometry. Profilometry is a gold standard method to assess tooth wear in vitro and commonly used by many research groups (Paepegaey *et al.* 2013). The laser used in this experiment was a red light confocal laser with a spot size of 2 μm , a sensor resolution of 0.01 μm and repeatable to 0.2 μm over a 10 mm range was chosen (Manufacturer's Instructions, Taicann Technologies, Southampton, UK). There are advantages and disadvantages to a laser spot size this small. The data obtained from the smaller spot size is very detailed and allowed us to capture very early erosive wear. However, a spot size of 2 μm can enter into an enamel prism and possibly increase the vertical step height measured. Experiment data obtained are not directly comparable to data obtained using another profilometer with a larger spot size or stylus head, although statistical relationships should remain the same (Paepegaey *et al.* 2013).

There is a direct conflict in the level of erosion needed to obtain accurate profilometry or accurate microhardness results. Microhardness analysis can quantify the softening of the exposed surface, but is not an indicator of enamel loss. The conditions of this model support the concept that the erosive process involves different levels of surface softening with enamel loss (Hara and Zero 2008; Venasakulchai *et al.* 2010). The microhardness data in this experiment were more qualitative than quantitative. Microhardness testing using this protocol may contrast with other studies investigating erosion where no profilometric loss has

occurred but support others reporting on more aggressive erosion models where profilometric tissue loss is likely to have occurred (Hannig and Balz 1999; Cheaib and Lussi 2011). The plateauing of microhardness after five cycles of erosion has been observed in other multiple cycling in vitro studies (Barbour *et al.* 2003; Venasakulchai *et al.* 2010). Rakhmatullina *et al.* observed a rapid linear loss of enamel hardness after 12-16 minutes of erosion with 0.65% citric acid at a pH of 3.6. After a certain point, the relationship between erosion and microhardness was not linear and hardness measurements plateaued (Rakhmatullina *et al.* 2013). For this reason microhardness testing is limited when assessing aggressive erosion unless reporting microhardness recovery with remineralisation (Hara and Zero 2008; Stenhagen *et al.* 2010).

Another interesting finding in this study was seemingly conflicting data between the microhardness and profilometry readings. Typically, an increased microhardness change would be associated with more erosion and therefore an increased profilometric loss. However, a statistically greater microhardness change was associated with the statistically lowest step height for one cycle of erosion. This means that although there has not been profilometric surface loss the remaining structure is softer. This softened structure could imply the presence of an intact enamel matrix, which may have the potential for remineralisation. Conversely, this softened enamel may be more susceptible to degradation from mechanical wear processes (Wiegand *et al.* 2013; Lussi *et al.* 2014). Schlueter *et al.* reported in her in vitro study investigating tin uptake into enamel that layers of stannous incorporated into the enamel varied in thickness. Unfortunately, no microhardness testing was performed but this newly formed matrix may have

been softer and potentially more protective against an erosive challenge (Schlueter, Hardt, *et al.* 2009). Further investigation and clinical studies are needed to answer this question.

2.7 CONCLUSION

Altering the timing of application of both stannous and sodium fluoride, either before or after an erosive challenge, resulted in statistically significant differences in erosion observed on enamel samples. Therefore the null hypothesis was rejected. Results from this study suggest that the effectiveness of fluoride in the reduction of erosive tooth wear might be explained by the timing of the fluoride application in addition to the type of fluoride used. This study may give an insight into the different mechanisms of action of two different fluorides as well as providing insight into their optimal clinical use. Further *in situ*, and ideally *in vivo*, work is required before clinical recommendations can be made. The additional objective for training and understanding the methods involved with profilometry was also accomplished

CHAPTER 3: QUESTIONNAIRE DEVELOPMENT AND VALIDATION

3.1 INTRODUCTION

The multi-factorial nature of dietary erosive tooth wear and interplay between risk factors pose challenges when attempting to capture a comprehensive risk pattern. To date, there are a limited number of questionnaires investigating dietary factors associated with erosive tooth wear. A recent meta-analysis investigating the relationship between dietary acids and tooth wear in children noted the lack of standardised formats for reporting dietary acid intake, particularly when reporting on frequency of dietary acid consumption (Salas *et al.* 2015).

The type of diet assessment has been observed to influence study outcome (Salas *et al.* 2015), which may be a reason for contradicting epidemiological studies regarding dietary acids. Some have shown strong associations between dietary acids and erosive tooth wear (Dugmore and Rock 2004a; Bartlett *et al.* 2013) and others have not (Chadwick *et al.* 2005; Alvarez Loureiro *et al.* 2015).

The aim of this chapter was to develop and validate a questionnaire, which could provide a comprehensive risk assessment for the pattern of dietary acid consumption. Examiner training for the basic erosive wear examination (BEWE) and assessment of intra-examiner reliability was also completed as part of this exercise.

3.1.1 OBJECTIVES

1. To calibrate the investigator to a gold standard examiner in the Basic Erosive Wear Examination (BEWE), assessing inter- and intra-examiner correlations.
2. To develop a questionnaire assessing dietary acid intake.
3. To assess the questionnaire using content validity, discriminatory validity and test-retest reliability.

Based upon a review of the literature, the objective of the questionnaire was to record the following information:

1. The daily frequency of dietary acid intake.
2. Consumption of dietary acids with and between meals.
3. The time taken to consume fruit and acidic beverages.
4. Drinking habits present prior to swallowing (sipping, swishing or holding drinks in the mouth).
5. Different types of containers (cup, glass, bottle, can) which are used when consuming acidic drinks.
6. Type of tooth brush, the amount of time spent brushing teeth and the tooth paste used.
7. The timing of tooth brushing in relation to meals.
8. Whether tooth brushing is regularly performed within 10 minutes of an erosive challenge.
9. Age, gender and self-reported dental hypersensitivity.

A further objective was to exclude participants with potential intrinsic erosion aetiology within the questionnaire.

3.2 METHODS

3.2.1 BEWE TRAINING AND INTER-EXAMINER RELIABILITY

The Basic Erosive Wear Examination (BEWE) index (Bartlett *et al.* 2008) was selected to grade erosive tooth wear. The clinical investigator was trained and calibrated in the use of the BEWE by a gold standard examiner (DB).

This index uses a 0-3 ordinal scale which grades the percentage of surface area affected by erosive wear (Figure 19).

Figure 19: BEWE Criteria for grading erosive wear (Bartlett et al. 2008)

0	No erosive wear
1	Initial loss of surface texture
2*	Distinct defect, hard tissue loss <50% of the surface area
3*	Hard tissue loss ≥50% of the surface area

* In scores 2 and 3 dentine often is involved

Initial training took place on study models. Ten sets of study models were examined without magnification and graded separately on the buccal, occlusal and palatal/lingual surfaces of each tooth excluding third molars. Teeth showing restorations involving >50% of the tooth, traumatised or carious teeth were excluded. A sextant BEWE score was allocated by recording the highest wear score present in each sextant. The total BEWE score was calculated by adding the sum of each sextant BEWE score, which could range from 0-18. Each investigator performed the examination separately and was blinded to the scores of the other examiner. Inter-examiner reliability on study models was assessed at sextant level (n=60).

Following this, training took place on patients. Patients were approached and asked to participate in a brief calibration training exercise. Verbal consent was obtained from ten patients to undertake a basic erosive wear examination. No patients refused to participate. Examinations were carried out under normal dental surgery conditions with the patient in a reclined position and good lighting. The teeth were dried and cleaned with compressed air. All teeth, excluding third molars, were examined using the same process as described above. Both examiners separately scored the highest BEWE score in each sextant whilst being blinded to the results of the other examiner. Inter-examiner reliability on patients was analysed at sextant level (n=60).

3.2.2 *QUESTIONNAIRE DEVELOPMENT*

Previously validated questionnaires within the field of dental erosion and other areas within healthcare were assessed.

3.2.2.1 Excluding intrinsic erosion aetiological factors.

As far as possible, other potential causes of erosive tooth wear were excluded.

Participants were excluded if they suffered regularly from heartburn, vomiting, chest pain, regurgitation, dry mouth or uncontrolled parafunctional habits such as clenching or grinding. However, participants with dental erosion as the primary wear aetiological factor, evidenced clinically by crater lesions, cupping on the dentition or loss of surface morphology, which did not correspond to the opposing dental surfaces, were included. The medical notes were checked to verify that the patient did not report any diseases likely to cause dental erosion such as eating disorders, diagnosed gastro-oesophageal reflux, prescription for heartburn or any known xerostomic medication.

3.2.2.2 Demographic details and self-reported hypersensitivity

Age and gender data were obtained. Participants were asked if they regularly suffered from dental hypersensitivity. If confirmed, participants were questioned which stimuli caused sensitivity; hot things, cold things/drinks/ice, sweet things, cold weather, brushing or other (N.X. West *et al.* 2013). Participants were asked to grade the pain from their sensitive teeth on a numeric pain rating scale of 0-10. Clinical dental hypersensitivity was not assessed for feasibility reasons.

3.2.2.3 Assessing dietary acid intake patterns

The purpose of this questionnaire was to capture all dietary acid consumption and not limit the assessment to individual dietary items. For fruits, the questionnaire focused on specific high-risk dietary acids e.g. citrus fruits or those readily available in the United Kingdom (apples, grapes and berries). All other fruits were then grouped together. To assess acidic drinks, fruit juices and carbonated beverages were analysed separately. All other acidic drinks as reported in the literature (Wang and Lussi 2012) were grouped together. If there was uncertainty whether a reported beverage was acidic, it was excluded.

Participants were questioned on the daily frequency of dietary acid consumption. Participants were also questioned on dietary acids consumed “less than once a day but greater than once a week”. For each dietary acid reported, participants were asked whether it was consumed with a meal or between meals and whether they consumed it over a period less than 5 minutes, between 5 and 10 minutes or greater than 10 minutes. For acidic drinks, participants were asked if they sipped, swished or held the drinks in the mouth prior to swallowing and whether they frequently drank the drink from a cup, a glass, a bottle or a can. In addition, they were asked if they regularly used a straw.

3.2.2.4 Assessing daily tooth brushing patterns

Participants were questioned whether the tooth brush they most often used was electric or manual. If manual, they were asked if they usually used a soft, medium or hard bristled tooth brush. Participants reported whether they used de-sensitising tooth paste and which tooth paste they used. Following this, participants were asked on the daily frequency of tooth brushing and the timing of this in relation to mealtimes. Time periods chosen were: less than ten minutes, between ten minutes and one hour and greater than one hour before or after eating.

Assessment of tooth brushing within 10 minutes of consuming something acidic was performed using two questions:

1. Participants were questioned whether they regularly had fruits, citrus fruit or juice for breakfast. This information was used with previously obtained information about the timing of tooth brushing in relation to breakfast, to assess if the patient brushed before or after a dietary acid for breakfast.
2. Participants were directly asked if they regularly brushed their teeth within 10 minutes of consuming a dietary acid. Participants were allowed to seek clarification over what constituted as a dietary acid.

3.2.2.5 Content validity

Following selection of the items to be examined, content validity was established through consensus with two expert senior clinical researchers in the tooth wear field (Professor David Bartlett and Dr. Rebecca Moazzez).

3.2.2.6 Formatting the questionnaire

Due to the complexity of data needed and possible need for explanation of dietary acids, the decision was made to collect data via an interviewer-led questionnaire. A structured conversational interviewing technique was to be used.

The questionnaire was formatted with a statistician to ensure that data input was optimal for analysis and able to facilitate combining and interacting separate risk factors.

3.2.2.7 Piloting the questionnaire

Following development of the questionnaire, the questionnaire was pre-tested for comprehension and legibility on a group of ten colleagues. The clinical investigator interviewed each volunteer. From this, data were collected about the questions most likely to cause confusion or give variable answers. Slight alterations to wording and standardised formats were composed for clarification if multiple answers were possible. If patterns varied between different days for participants, what the patient did most regularly was recorded. Participants had the opportunity to clarify if food items counted as a fruit (e.g. tomatoes) and if certain drinks were considered acidic. Attempts to minimise interviewer bias were made by adopting a neutral tone and no feedback was given to the participant to reduce social desirability bias.

Following feedback and alterations to the questionnaire wording from the first set of piloting, the questionnaire was piloted on a group of patients attending care planning clinics in King's College London Dental Institute. Verbal consent was obtained for participation and feedback was obtained regarding comprehension and legibility. For this group, the questionnaire was timed. Each questionnaire lasted between 5-9 minutes depending on the complexity of the participant's

dietary acid consumption. This was deemed as acceptable to the participants involved. Following this second set of piloting and feedback, the questionnaire was subjected to the validation process.

3.2.3 *DISCRIMINANT VALIDITY*

Discriminant validity is defined as the ability of the questionnaire to discriminate between two groups. The questionnaire was assessed to determine if it could detect statistical differences in the daily frequency of dietary acid consumption between a cohort of patients with severe erosive tooth wear (n=25) and controls (n=25). The source population were adults aged 18 years or older who were referred by their GDP into specialist restorative clinics at Guy's Hospital, King's College London Dental Institute for either tooth wear (erosive wear patients) or other treatment (control group).

Erosive wear patients were defined as those with a BEWE score of 12 or higher and at least one score of 3 in a sextant. The exclusion criteria were: no missing anterior teeth or anterior crowns/bridges or implants and a minimum of 10 teeth in the upper and 10 teeth in the lower jaw. A history of eating disorders, gastro-oesophageal reflux, xerostomia, bruxism, prescribed xerostomic/heartburn medication, pregnancy, involvement in other research within the past 30 days or inability to speak or understand the English language also excluded the participant from this study. They were also excluded if there was the presence of caries on more than one tooth.

Controls were defined as those with a BEWE score of 10 or lower and could not have a score of 3 on any surface of any tooth. Apart from the clinical diagnosis, the

same inclusion and exclusion criteria applied to the controls. A list of the inclusion and exclusion criteria can be seen in Appendix Section 7.1.

Ethical approval was obtained to conduct the study (REC ref 14/WS/0015). After diagnosis by their referring dentist for severe erosive wear or no/mild tooth wear, participants were invited to take part in a diet questionnaire-based study and basic erosive wear examination. Participants were advised that they were under no obligation to participate, and following this informed written consent was obtained. Participants were then asked the questionnaire by the clinical investigator and a BEWE examination was performed as described in section 3.2.1

3.2.4 *TEST-RETEST RELIABILITY AND INTRA-EXAMINER RELIABILITY*

Test-retest and intra-examiner reliability were tested simultaneously. Participants, due to return for additional visits with their treating dentist, were invited to repeat the procedure at their follow up appointment. Following agreement, the examination and questionnaire were repeated at their follow up appointment after a minimum two week interval period. The clinical investigator was blinded to the results of the previous appointment.

Intra-examiner reliability was assessed at the BEWE sextant level and patient level (total BEWE score). As there was large variability in the types of dietary acids consumed between participants, the questions with the greatest number of responses were chosen to perform test-retest reliability. These were: daily frequency of dietary acid intake, daily frequency of fruit intake, time taken to consume fruit, daily frequency of acidic drink intake, time taken to consume acidic drinks and whether the patient sipped, swished or held acidic drinks in the mouth prior to swallowing.

3.3 STATISTICS

All analyses were performed using IBM SPSS Statistics 22 (IBM Corporation, Armonk, New York) apart from weighted kappa analysis which was performed in Stata vers. 14 (Statacorp, College Station, TX: Statacorp LP.)

For discriminant validity, data were assessed for normality using normality plots and Shapiro-Wilks tests. As the data were not normally distributed, Mann Whitney U-tests were used to assess for differences between the two groups.

For inter- and intra-examiner and test-retest reliability, Kappa scores were analysed for categorical variables. Weighted Kappa scores were analysed for ordinal variables and absolute agreement intra-class correlation coefficients (ICC's) were determined for continuous data. Inter-examiner and intra-examiner percentage agreement for each BEWE score was also assessed and reported upon. The value of Kappa, identifying the strength of agreement, was categorised according to Masson *et al.* as follows: <0.20: poor, 0.21- 0.40: fair, 0.41- 0.60: moderate, 0.61- 0.80: good, 0.81- 1.00: very good (Masson *et al.* 2003).

Interpretation of the ICC was based according to Cicchetti 1994 and are defined as follows: <0.40: poor, 0.40-0.59: fair, 0.60-0.74: good, 0.75-1.00: excellent (Cicchetti 1994). For all analyses, a p-value <0.05 was accepted as significant.

3.4 RESULTS

3.4.1 INTER-EXAMINER TRAINING

Figure 20 presents the percentage agreement for each BEWE score between DB and SOT. Agreement was higher when assessment was performed on patients compared to study models. The highest agreement was on the absence of erosive tooth wear (score 0, 100% agreement) and was followed by the most severe score (score 3, 94.4% agreement). The lowest agreement was for moderate erosive tooth wear present on casts (score 2, 65.4 % agreement). However, agreement increased to 88.9% when examined on patients.

Inter-examiner Kappa scores between DB and SOT were good (0.72) when assessing study models and very good (0.85) when assessing patients. Figure 20 reports the kappa values in addition to the percentage agreement between DB and SOT for each BEWE sextant score.

Figure 20: Inter-examiner training results reporting kappa scores and inter-examiner agreement

BEWE Score	Casts (Kappa = 0.72)	Patients (Kappa = 0.85)
0	100%	100%
1	86%	87%
2	65.4%	88.9%
3	82.1%	94.4%

3.4.2 INTRA-EXAMINER RELIABILITY

Reassessment of 29 patients (n=174 sextants) by the same examiner (SOT) resulted in a kappa score of 0.75 (good) for the BEWE sextant score and an ICC of 0.96 (95% CI 0.90 – 0.98, excellent) for the total BEWE score. Figure 21 below reports the percentage agreement on each BEWE score taken at the two separate appointments. There were no sextants observed with complete absence of wear in this adult sample (BEWE score 0). The highest agreement was score 1 (mild erosive tooth wear), followed by 3 (severe erosive wear).

Figure 21: Intra-examiner agreement at sextant level (n=174)

BEWE score	Percentage agreement between each visit
1	100%
2	82.4%
3	90.5%

3.4.3 DISCRIMINANT VALIDITY

The median frequency intake of daily dietary acid consumption was 4 (IQR 3, 6 [Range 2-8]) for the erosive wear group and 2 for the control group (IQR, 0, 3 [Range 0-5]). This difference was statistically significant ($p < 0.001$).

3.4.4 TEST-RETEST RELIABILITY OF QUESTIONNAIRE

The daily frequencies of total dietary acid consumption, daily fruit consumption and daily acidic drink consumption were significantly correlated ($p < 0.001$). The ICC values are reported in Figure 22. All variables demonstrated excellent ICC values (> 0.75).

Figure 22: Test-retest reliability ICC values for daily frequency of dietary acid consumption

Variable	ICC	95% CI
Daily frequency of fresh fruit consumption	0.84	(0.66-0.93)
Daily frequency of acidic drink consumption	0.80	(0.58-0.91)
Frequency of daily dietary acid consumption	0.85	(0.68-0.93)

Weighted Kappa scores for the time taken to consume fruit, time taken to consume acidic drinks and self-reported sipping, swishing or holding the drinks in the mouth are reported in Figure 23. Correlations were not as strong for categorical variables although are still classified as moderate (time taken to consume acidic drinks: Kappa = 0.59) to good (time taken to consume fruit, sipping, swishing or holding drinks in the mouth: Kappa = 0.61).

Figure 23: Weighted Kappa values for test-retest reliability of categorical variables

Variable	Weighted Kappa	p value
Time taken to consume fruit	0.65	$p < 0.001$
Time taken to consume acidic drinks	0.59	$p = 0.018$
Sipping, swishing or holding drinks in the mouth	0.61	$p = 0.001$

Overall the data suggest good to excellent levels of agreement between visits within the same participant. The time taken to complete the questionnaire and the examination remained at 5-10 minutes which was deemed to be acceptable to the participant and the interviewer.

3.5 DISCUSSION

The present exercise describes the development and validation of an interviewer-administered questionnaire assessing patterns of dietary acid consumption. BEWE training and calibration were also performed. The BEWE was chosen as the index to be used to discriminate between the two groups of patients. This index has been developed by expert consensus (Bartlett *et al.* 2008), has been previously validated (Olley *et al.* 2014), is deemed to have sufficient specificity and sensitivity when compared to other indices (Mulic *et al.* 2010; Margaritis *et al.* 2011; Dixon *et al.* 2012) and is a relatively quick index designed for both general practice and epidemiological studies.

Inter-examiner agreement was higher when the BEWE examination was performed on patients than on study models. This has been observed in other studies (Mulic *et al.* 2010; Dixon *et al.* 2012) or when clinical photography is used as an adjunct (Mulic *et al.* 2010). This may reflect the importance of subtle changes in the texture and tooth surface, which is not detectable on study models and should be taken into consideration if training is limited to study models in epidemiology studies or when monitoring tooth wear in vivo.

The inter-examiner kappa scores on patients were higher in this study (Kappa = 0.85) when compared to those reported in other studies (Mulic *et al.* 2010; El Aidi *et al.* 2011; Bartlett *et al.* 2013). This may have been the result of the individualised

training as it was only necessary to calibrate a single examiner. The intra-examiner correlations were also high. The high levels of both intra and inter examiner agreement observed are important given that inclusion/exclusion criteria for further clinical studies within this thesis are based upon the presence/absence of BEWE score 3.

The judgement of what constituted as severe erosive wear was based on an arbitrary decision but influenced by experience and previous work. The presence of a BEWE score of 3 to represent severe wear as participants, particularly the elderly, may show moderate signs of wear in all sextants without it being pathological (Donachie and Walls 1996). The decision to combine this with a total BEWE score of 12 was thought to be a balance between representing severe wear and not including too strict criteria which would negatively influence recruitment. On balance, it was felt that the two criteria represented severe wear.

A range of statistical approaches to evaluate the validity of the questionnaire were used, due to the absence of a gold standard questionnaire for dietary assessment of erosive wear. Content validity was established with a review of the literature and experts in the field. Discriminant validity data confirmed the questionnaire to be capable of discriminating between a group with severe erosive tooth wear and mild/moderate tooth wear when assessing the total frequency of fruit and acidic drink intake. The median daily frequency of fruit or acidic drink intake of those with severe erosive tooth wear found in this study was 4. When comparing results from other studies, one longitudinal study reported that the group with a high rate of tooth wear progression were found to have 4 or greater dietary acid intakes per day (Lussi and Hellwig 2014). O'Sullivan and Curzon found that 3+ intakes of

acidic drinks per day resulted in increased risk of severe erosion (O'Sullivan and Curzon 2000). Test-retest reliability was high when assessing frequency of dietary acid consumption.

Correlations were markedly lower when reporting the time taken to consume dietary acids and the presence of an alternative drinking method prior to swallowing (Kappa scores 0.59-0.65). Although these results are classified as moderate to good (Masson *et al.* 2003), the reduced values may be a reflection of the regularity of the occurrence. Events that happen regularly are easier to recall and subject to less reporting error than events which do not happen regularly (Menon 1993). Although an individual may have a dietary acid every day they may not consume it over the same time period every day or in the same way every day. This may introduce an inherent bias (availability heuristic) whereby the most recent behaviour example is reported as it is more accessible by memory (Gilovich *et al.* 2002) leading to error.

This questionnaire did not assess the quantity of dietary acid intake. Capturing quantity of food and beverage data is difficult for reasons outlined in the literature review section 1.2.1.1. Capturing the quantity of intake was attempted during the piloting stage of questionnaire development. However, portion sizes in fruit and drinks varied largely between individuals, in addition to the quantity, which they reported to consume. This has also been found in other large epidemiology studies (Andersen *et al.* 2004). There is little guidance in the literature when attempting to quantify small amounts of frequent consumption for example a sip of a drink, or a small bite/segment of fruit. It was deemed more important to capture the frequency at which the participant sipped the drink/had a small piece of fruit

rather than attempt to estimate the quantity ingested. This is an area of the questionnaire which could be improved upon; a recent meta-analysis observed that the overall quantity and not the frequency was a more significant predictor for caries disease development (Bernabe *et al.* 2016). There are no studies comparing frequency of dietary acid intake to quantity of acid consumed as a risk factor in erosive tooth wear.

3.6 CONCLUSION

The questionnaire to be used within this thesis demonstrated content validity, discriminant validity and test-retest reliability. The clinical investigator demonstrated very good agreement with a gold standard investigator when performing the basic erosive wear examination. Intra-examiner agreement was high. This questionnaire is limited in measuring the quantity of dietary acids consumed.

CHAPTER 4: RETROSPECTIVE CASE-CONTROL STUDY

INVESTIGATING THE TIMING OF ORAL HYGIENE PROCEDURES, DIETARY ACID INTAKE AND EROSIVE TOOTH WEAR.

4.1 OVERVIEW

Previous epidemiological studies have investigated individual dietary risk factors rather than overall patterns of consumption and habits. While each factor is important, it may be a combination that determines whether the tooth wear progresses. There is little clinical data supporting the consumption of dietary acids taken with meals. The interactive role of how erosion and abrasion interact is also unclear (Bartlett *et al.* 2013). This study aims to utilise a questionnaire-based case-control methodology to capture risk patterns associated with erosive tooth wear and investigate the relative risk surrounding each effect.

4.2 OBJECTIVE

To assess the interrelationship between dietary acid consumption behaviours, timing of tooth brushing and erosive tooth wear.

4.3 NULL HYPOTHESIS

1. There will be no association between the frequency of dietary acid intake and severe erosive tooth wear.
2. There will be no association between erosive tooth wear and the duration of consumption of dietary acids.
3. There will be no association between severe erosive tooth wear and the timing of tooth brushing to meals or dietary acid consumption.

4.4 METHODS

This was a single-centre, frequency-matched, case-control study. The study protocol was approved by West of Scotland Research Ethics Service (Reference 14/WS/0015) and written informed consent was obtained from all participants. The present study adhered to the Strengthening the Reporting of Observational Studies (STROBE) statement (Vandenbroucke *et al.* 2007) and is registered at clinicaltrials.gov (Identifier number: NCT02449434).

4.4.1 PARTICIPANTS

Participants (n=600) aged 18 years or older, were recruited between May 2014 and March 2016 following referral by their general dental practitioners (GDP) for erosive tooth wear (n=300) or general treatment (controls, n=300) to restorative clinics at King's College London Dental Institute.

Inclusion/exclusion criteria were the same as described for the validation exercise in the previous chapter (Appendix 8.1). From the pilot study a minimum sample size of 490 participants (245 in each group) were needed. This calculation assumed the proportion of adults with high dietary acid intake (3+ times/day) was 55% among cases and 40% among controls (expected odds ratio of 2.25), case-control ratio of 1-to-1, 90% statistical power and 95% significance level. A previous cross-sectional study within our group (Bartlett, Fares, *et al.* 2011) on a convenience sample of 1,010 adults demonstrated odds ratios of 1.4 and 5 for fresh fruit intake and drinking method respectively. Based on these data, a prediction was made that a cohort of 300 patients with severe erosion and 300 controls would show statistical differences for less common risk factors.

4.4.2 DATA COLLECTION

Data collection procedures were identical for cases and controls. The Basic Erosive Wear Examination (BEWE) index graded tooth wear on the buccal, occlusal and palatal/lingual surfaces of each tooth excluding third molars and was used to differentiate the groups as described in the previous chapter. A single trained and calibrated examiner (SOT) carried out all clinical examinations in a dental chair with good lighting and after drying the teeth with compressed air. Erosive tooth wear cases were defined as those with a BEWE score of 12 or higher and at least one score of 3 in a sextant whereas controls were defined as those with a BEWE score of 10 or lower and no score of 3 on any surface of any tooth (clinically classified as no or mild erosive tooth wear). Controls were frequency age-matched on a 1:1 ratio with cases over six age groups (18-25, 26-35, 36-45, 46-55, 56-65 and 66+ years). Cases that could not be matched were excluded from the study.

After recruitment, a trained interviewer (SOT) used the previously validated questionnaire to assess the participants' potential risk factors. Participants were asked about the frequency, timing of consumption (with meals or between meals) and duration of consumption of fruits, fruit drinks, carbonated beverages and other acidic drinks, the type of holder (cup, glass, bottle, can) and whether they had an alternative drinking method (sipping, swishing or holding drinks in the mouth) prior to swallowing. In addition, tooth brushing routines were assessed and participants were questioned on whether they usually brushed within 10 minutes of consuming something acidic. Age, gender and self-reported hypersensitivity data were also captured.

4.4.3 *DATA ANALYSIS*

All analyses were performed in the IBM SPSS Statistics 22 (IBM Corporation, Armonk, New York). Numbers of cases in each category were assessed. If containing less than 5% of total participants (n=30) the category was collapsed or the variable was excluded from analysis. The daily frequency of both fruit and acidic drink consumption were summed to give a total daily frequency of dietary acid consumption.

Erosive wear patients and controls were initially compared using the Chi-square test for categorical variables and the t-test for continuous measures. Following the observation that gender was a potential confounding factor, risk factors were analysed using unconditional binary logistic regressions and reported using odds ratios (OR) adjusting for sex and age group, using presence or absence of severe erosive wear as the dependent variable. Variables included in the multivariate logistic regression model were manually selected based upon prior theory, the research hypothesis and statistical significance.

4.5 QUESTIONNAIRE RESULTS

Tables reporting the raw frequencies and crude analysis results not reported here can be observed in Appendix Section 8.3 of this thesis. Presented here are demographics and results relating to the research hypothesis.

Figure 24 reports the demographic and clinical characteristics of erosive wear patients and controls. More males ($n=162$) presented with erosive wear than females ($n=138$) and this difference was statistically significant ($p=0.003$). As this statistical difference was observed, it was controlled for in subsequent analysis. Frequency age matching was effective as demonstrated by the lack of statistical differences between groups. The overall prevalence of self-reported hypersensitivity was high (45.3%). However, a statistically greater number of erosive wear patients reported to be currently suffering from sensitive teeth than controls ($n=166$, and $n=106$ respectively, $p<0.001$).

Figure 24: Demographic and clinical characteristics of erosive wear patients and controls

		Erosive Wear Patients n (%)	Controls n (%)	p value
Gender	Males	162 (54%)	125 (41.7%)	0.003
	Females	138 (46%)	175 (58.3%)	
Age	18-25	32 (10.3%)	32 (11%)	1
	26-35	67 (22.3%)	67 (22.3%)	0.837
	36-45	67 (22.7%)	67 (22%)	0.761
	46-55	66 (22%)	66 (22%)	0.837
	56-65	44 (14.7%)	44 (14.7%)	0.849
	66+	24 (8%)	24 (8%)	0.870
Age in years	Mean \pm SD	44.07 \pm 14.17	43.76 \pm 14.71	0.79
	Range	18-74	18-75	
BEWE score	Mean \pm SD	15.01 \pm 2.30	6.27 \pm 2.79	<0.001
	Range	12-18	0-10	
Self-reported sensitive teeth	No	134 (44.7%)	194 (58.3%)	<0.001
	Yes	166 (55.3%)	106 (41.7%)	

4.5.1 DAILY TOOTH BRUSHING BEHAVIOURS

Figure 25 compares the daily tooth brushing habits of erosive wear patients and controls. No large disparities in numbers were observed between the two groups although differences were observed. A comparatively small number of participants brushed their teeth using a hard manual toothbrush (n=38), however a greater number of them were patients with erosive wear (n=29, OR 3.19, 95% CI: 1.44 – 7.06, p=0.004). In addition, fewer controls (n=11) brushed their teeth after lunch compared to erosive wear patients (n=28, OR 2.73, 95% CI: 1.32 – 5.62, p=0.007). Interestingly, a greater number of controls (n=71) allowed 10 minutes or greater to pass before brushing their teeth compared to erosive wear patients (n=42) and a small protective association for controls was observed (OR 0.55, 95% CI: 0.35 –

0.86, $p=0.009$). Although not significant, there was a trend that brushing teeth offered a protective effect. A greater number of erosive wear patients did not brush their teeth at breakfast time (erosive wear patients: $n=19$, controls: $n=9$) and at dinner time (erosive wear patients: $n=43$, controls: $n=33$).

Figure 25: Daily tooth brushing behaviours

Variable	Erosive Wear Patients n (%)	Controls n (%)	OR	95% CI	p value
Type of toothbrush used					
Medium manual	135 (45%)	126 (42%)	1		
Soft manual	34 (11.3%)	25 (8.3%)	1.26	(0.69-2.29)	0.453
Hard manual	29 (9.7%)	9 (3%)	3.19	(1.44-7.06)	0.004*
Electric toothbrush	102 (34%)	140 (46.7%)	0.74	(0.52-1.05)	0.090
Time spent brushing teeth					
≥2 min	244 (81.3%)	237 (79%)	1		
<2 min	56 (18.7%)	63 (21%)	0.86	(0.57-1.29)	0.46
Frequency of daily tooth brushing					
Once or less than once daily	49 (16.4%)	34 (11.3%)	1		
2/day	225 (75%)	253 (84.3%)	0.67	(0.41 -1.08)	0.101
3+ /day	26 (8.6%)	13 (4.3%)	1.52	(0.68 -3.39)	0.309
Brushing before/after breakfast					
Brushes before breakfast	151 (50.3%)	136 (45.3%)	1		
Brushes <10 min after breakfast	88 (29.3%)	84 (28%)	0.97	(0.66 - 1.42)	0.861
Brushes ≥ 10 min after breakfast	42 (14%)	71 (23.7%)	0.55	(0.35 - 0.86)	0.009*
Does not brush at breakfast	19 (3.2%)	9 (1.5%)	1.69	(0.74 - 3.91)	0.216
Does the patient brush after lunch?					
No	272 (90.6%)	289 (96.3%)	1		
Yes	28 (9.4%)	11 (3.7%)	2.73	(1.32-5.62)	0.007*
Does the patient brush after dinner?					
Does not brush after dinner	46 (15.3%)	33 (11%)	1		
Brushes < 10 min after dinner	30 (10%)	35 (11.7%)	0.63	(0.32 -1.23)	0.173
Brushes ≥ 10 min after dinner	224 (74.7%)	232 (77.3%)	0.73	(0.44 - 1.19)	0.202

4.5.2 TIMING OF TOOTH BRUSHING AND DIETARY ACID INTAKE

A greater number of patients with erosive wear self-reported as brushing within 10 minutes of consuming a dietary acid (n=95 and controls: n=56, OR 2.2, 95% CI: 1.46-3.17, p<0.001).

Figure 26: Self-reported tooth brushing within 10 min of consuming a dietary acid

	Erosive Wear Patients n (%)	Controls n (%)	OR	95% CI	p value
Does the patient brush within 10 min of consuming a dietary acid?					
No	205 (68.3%)	244 (81.3%)			
Yes	95 (31.7%)	56 (18.7%)	2.20	(1.46 - 3.17)	<0.001*

Figure 27 reports the timing of tooth brushing in relation to consuming dietary acids at breakfast. More erosive wear patients (n=155) consumed dietary acids for breakfast compared to controls (n=125, OR 1.6, 95% CI: 1.15 – 2.22, p= 0.005). A greater number of erosive wear patients brushed their teeth within 10 minutes of consuming juice for breakfast although the overall numbers of participants who did this was small (erosive wear patients, n=27 and controls n=14, OR 2.69, 95% CI: 1.06 – 6.82, p=0.036).

Figure 27: Timing of tooth brushing in relation to consuming dietary acids at breakfast

	Erosive Wear Patients n (%)	Controls n (%)	OR	95% CI	p value
Does the patient consume fruits, citrus or juice for breakfast?					
No	145 (48.3%)	175 (58.3%)	1		
Yes	155 (51.7%)	125 (41.7%)	1.6	(1.15-2.22)	0.005*
Of those that consume dietary acid for breakfasts					
Either fruit, citrus or juice	n=155	n=125			
Brushes before breakfast	75 (48.4%)	56 (44.8%)	1		
Brushes < 10 min after	55 (35.5%)	39 (31.2%)	1.08	(0.62 – 1.87)	0.787
Brushes ≥ 10 min after	18 (11.6%)	26 (20.8%)	0.52	(0.26 - 1.06)	0.071
Does not brush at breakfast	7 (4.5%)	4 (3.2%)	1.05	(0.29 – 3.87)	0.941
Juice for breakfast	n=68	n=54			
Brushes before breakfast					
Brushes < 10 min after	30 (44.1%)	31 (57.4%)	1		
Brushes ≥ 10 min after	27 (39.7%)	14 (25.9%)	2.69	(1.06 – 6.82)	0.036*
Does not brush at breakfast	9 (13.2%)	7 (13%)	1.73	(0.52 – 5.80)	0.373
	2 (2.9%)	2 (3.7%)	0.68	(0.07 – 6.84)	0.740
Citrus for breakfast	n=52	n=16			
Brushes before breakfast					
Brushes < 10 min after	26 (50%)	8 (50%)	1		
Brushes ≥ 10 min after	20 (38.5%)	6 (37.5%)	1.06	(0.29 – 3.95)	0.926
Does not brush at breakfast	2 (3.8%)	2 (12.5%)	0.31	(0.03 – 2.94)	0.310
	4 (7.7%)	0 (0%)	-		
Fruits for breakfast	n=89	n=74			
Brushes before breakfast	42 (47.2%)	31 (41.9%)	1		
Brushes < 10 min after	30 (33.7%)	22 (29.7%)	0.97	(0.47 - 2.02)	0.935
Brushes ≥ 10 min after	13 (14.6%)	19 (25.7%)	0.50	(0.21 – 1.20)	0.119
Does not brush at breakfast	4 (4.5%)	2 (2.7%)	1.43	(0.23 – 8.76)	0.699

4.5.3 BEHAVIOURS ASSOCIATED WITH CONSUMING DIETARY ACIDS

More erosive wear patients consumed fruit over periods >10 minutes on a daily basis (n=54 and controls n=14, OR 5.46, 95% CI 2.90-10.30, p<0.001) and consumed acidic drinks over periods > 10 minutes on a daily basis (n=142 and controls n=66). The strength of association, as determined by the odds ratios, was not as strong for the duration spent consuming acidic drinks although a statistically significant linear relationship was observed (Figure 28).

Figure 28: Duration over which dietary acids are consumed

Variable	Erosive Wear Patients n (%)	Controls n (%)	OR	95% CI	p value
Duration of fruit consumption daily					
Does not eat fruit daily	44 (14.7%)	54 (18%)			
<5 min	171 (57%)	203 (67.7%)	1		
5-10 min	56 (18.7%)	29 (9.7%)	1.36	(0.77-2.40)	0.284
>10 min	54 (18%)	14 (4.7%)	5.46	(2.90-10.30)	<0.001*
Duration of acidic drink consumption daily					
Does not drink acidic drinks daily	42 (14%)	136 (45.3%)			
<5 min	62 (20.7%)	68 (22.7%)	1		
5-10 min	54 (18%)	30 (10%)	2.14	(1.21-3.80)	0.009*
>10 min	142 (47.3%)	66 (22%)	2.56	(1.61-4.06)	<0.001*

A greater number of erosive wear patients (n=99) compared to controls (n=11) reported alternative drinking behaviours prior to swallowing acidic drinks, such as sipping, swishing or holding the drinks in the mouth which resulted in greatly increased odds ratios (9.32, 95% CI 4.78-18.18, p<0.001). Figure 29 overleaf reports the relationship between each of sipping, swishing or holding drinks in the mouth prior to swallowing and erosive tooth wear.

Figure 29: Relationship between sipping, swishing or holding drinks in the mouth prior to swallowing and erosive tooth wear

Variable	Erosive Wear Patients n (%)	Controls n (%)	OR	95% CI	p value
Any alternative drinking behaviour prior to swallowing					
Does not drink acidic drinks	42 (14%)	136 (45.3%)			
Drinks acidic drinks but does not sip/swish/hold drinks	159 (53%)	153 (51%)	1		
Drinks acidic drinks and sips/swishes/holds drinks	99 (33%)	11 (3.7%)	9.32	(4.78-18.18)	<0.001*
Sip					
Does not drink acidic drinks	42 (14%)	136 (45.3%)			
Drinks acidic drinks but does not sip drinks	201 (67%)	162 (54%)	1		
Sips acidic drinks	57 (19%)	2 (0.7%)	6.93	(3.05-15.72)	<0.001*
Swish					
Does not drink acidic drinks	42 (14%)	136 (45.3%)			
Drinks acidic drinks but does not swish drinks	231 (77%)	162 (54%)	1		
Swishes acidic drinks	27 (9%)	2 (0.7%)	9.80	(2.29-42.00)	<0.001*
Hold					
Does not drink acidic drinks	42 (14%)	136 (45.3%)			
Drinks acidic drinks but does not hold drinks in the mouth	239 (79.7%)	161 (53.7%)	1		
Holds acidic drinks in the mouth	19 (6.3%)	3 (1%)	4.47	(1.29-15.48)	0.018*

The holder from which acidic drinks were consumed also appeared to have relationship with erosive wear (Figure 30). More erosive wear patients drank acidic drinks from a bottle on a daily basis (n=61) compared to controls (n=21) and this was statistically significant (OR 2.13, 95% CI 1.23-3.68, p=0.007).

Figure 30: Relationship between drinks container and erosive tooth wear

Variable	Erosive Wear Patients n (%)	Controls n (%)	OR	95% CI	p value
Does not drink acidic drinks	42 (14%)	136 (65.7%)			
Drinks acidic drinks daily but not from a bottle	197 (65.7%)	143 (47.7%)	1		
Drinks acidic drinks from a bottle daily	61 (20.3%)	21 (7%)	2.13	(1.23 – 3.68)	0.007*
Does not drink acidic drinks	42 (14%)	136 (45.3%)			
Drinks acidic drinks daily but not from a can	209 (69.7%)	134 (44.7%)	1		
Drinks acidic drinks from a can daily	49 (16.3%)	30 (10%)	1.05	(0.63-1.75)	0.857

4.5.4 FREQUENCY OF DIETARY ACID INTAKE

Figure 31 reports the relationship between dietary acid consumption and erosive wear. A greater number of erosive wear patients consumed ≥ 3 dietary acids on a daily basis (n=258) compared to controls (n=132). Further differences were observed when the timing of dietary acid intake was taken into consideration. More erosive wear patients consumed ≥ 3 dietary acids with meals (n=62) compared to controls (n=27) and odds ratios of 3.01 (95% CI: 1.82 – 4.95) were observed. A greater number of erosive wear patients (n=190) consumed the same

frequency of acid intake between meals compared to controls (n=59) and an odds ratios of 14.62 (95% CI 9.15 – 23.37) was observed. The odds ratio for ≥ 3 acidic challenges a day regardless of mealtimes was 14.17 (95% CI: 7.23-14.77).

Figure 31: Relationship between dietary acid consumption and erosive wear

	Erosive Wear Patients n (%)	Controls n (%)	OR	95% CI	p value
Daily frequency of fruit and acidic drink consumption irrespective of timing					
1 or less/day	11 (3.7%)	76 (25.3%)	1		
2/day	31 (10.3%)	92 (30.7%)	2.28	(1.07-4.88)	0.034*
3 or greater/day	258 (22%)	132 (26.3%)	14.17	(7.23-27.77)	<0.001*
Fruit and/or acidic drinks with a meal					
1 or less/day	168 (56%)	227 (75.7%)	1		
2/day	70 (23.3%)	46 (15.3%)	2.04	(1.33-3.12)	0.001*
3 or greater/day	62 (20.7%)	27 (9%)	3.01	(1.82-4.95)	<0.001*
Fruit and/or acidic drinks between meals					
1 or less/day	41 (13.7%)	164 (54.6%)	1		
2/day	69 (23%)	77 (25.7%)	3.82	(2.36-6.18)	<0.001*
3 or greater/day	190 (63.3%)	59 (19.7%)	14.62	(9.15-23.37)	<0.001*

When fruits and acidic drinks were analysed separately (Figure 32 overleaf), greater differences were observed in acidic drink intake patterns compared to fruit intake patterns between those with severe erosive wear and those without. Only 42 erosive wear patients (14%) did not drink acidic drinks on a daily basis compared to 136 (45.3%) of controls. Conversely, 197 erosive wear patients (65.7%) consumed two acidic drinks or more daily compared to 70 (23.3%) controls (OR 9.63, 95% CI: 6.10-15.19, $p < 0.001$). Although more erosive wear patients consumed 2 or greater fruit intakes daily ($n=187$ compared to $n=148$ for controls) the association was not as strong (OR 1.75, 95% CI 1.10 – 2.80, $p=0.019$).

When the timing of acid intake with meals and between meals were analysed separately, there were no significant differences between erosive wear patients and controls for fruit consumption with meals ($n=35$ and $n=40$, respectively, $p=0.309$). More erosive wear patients consumed their fruit between meals compared to controls (≥ 2 intakes between meals daily, erosive wear patients $n=156$, controls $n=74$, OR 4.14, 95% CI 2.68 – 6.37, $p < 0.001$). Similarly more erosive wear patients consumed their acidic drinks between meals (≥ 2 intakes between meals daily, erosive wear patients $n=140$, controls $n=36$, OR 10.75, 95% CI 6.72 – 17.18, $p < 0.001$). Interestingly, large increases in odds ratios were observed when the frequency of dietary acid consumption increased from once daily to twice daily across all groups, apart from fruit intake with a meal.

Figure 32: Fruit consumption and acidic drink consumption analysed separately

Variable	Erosive Wear Patients n (%)	Controls n (%)	OR	95% CI	p value
Daily frequency of fruit consumption					
Less than once daily	44 (14.7%)	54 (18%)	1		
1/day	69 (23%)	98 (32.7%)	0.91	(0.55-1.52)	0.730
2 or greater/day	187 (62.3%)	148 (49.3%)	1.75	(1.10-2.80)	0.019*
Fruits with a meal					
Less than once daily	191 (63.7%)	174 (58%)	1		
1/day	74 (24.7%)	86 (28.7%)	0.80	(0.55-1.17)	0.256
2 or greater/day	35 (11.7%)	40 (13.3%)	0.77	(0.46-1.28)	0.309
Fruits between meals					
Less than once daily	62 (20.7%)	107 (35.7%)	1		
1/day	82 (27.3%)	119 (39.7%)	1.25	(0.81 – 1.92)	0.310
2 or greater/day	156 (51%)	74 (24.7%)	4.14	(2.68 – 6.37)	<0.001*
Daily frequency of acidic drink consumption					
Less than once daily	42 (14%)	136 (45.3%)	1		
1/day	61 (20.3%)	94 (31.3%)	2.09	(1.30-3.38)	0.003*
2 or greater/day	197 (65.7%)	70 (23.3%)	9.63	(6.10-15.19)	<0.001*
Acidic drinks with a meal					
Less than once daily	130 (43.3%)	206 (68.7%)	1		
1/day	87 (29%)	75 (25%)	1.84	(1.25-2.70)	0.002*
2 or greater/day	83 (27.7%)	19 (6.3%)	7.12	(4.09-12.39)	<0.001*
Acidic drinks between meals					
Less than once daily	71 (23.7%)	186 (62%)	1		
1/day	89 (29.7%)	78 (26%)	3.20	(2.11-4.87)	<0.001*
2 or greater/day	140 (46.7%)	36 (12%)	10.75	(6.72-17.18)	<0.001*

4.5.5 *MULTIVARIATE ANALYSIS*

The variables chosen to be included in the multivariate analysis were manually selected based upon the research hypothesis and statistical significance. It was decided to perform two analyses. One multivariate analysis assessed the total daily frequency of dietary acid consumption with meals and between meals (represented by combined fruit and acidic drink intake, Figure 33), and another assessed fruit and acidic drink intake separately with meals and between meals (Figure 34). Other variables adjusted for were age, gender, self-reported brushing within 10 minutes of consuming a dietary acid, duration of fruit consumption, duration of acidic drink consumption and alternative drinking behaviours (including sipping, swishing or holding drinks in the mouth) prior to swallowing.

Figure 33: Multivariate analysis investigating daily frequency of dietary acid consumption and other risk factors

Variable	OR	95% CI	p value
Gender			
Male	1		
Female	0.40	(0.25 – 0.64)	<0.001*
Does the patient brush within 10 min of consuming a dietary acid?			
No	1		
Yes	1.48	(0.87 – 2.51)	0.147
Dietary acids with a meal			
1 or less/day	1		
2/day	3.00	(1.64-5.50)	<0.001*
3 or greater/day	3.99	(1.97-8.06)	<0.001*
Dietary acids between meals			
1 or less/day	1		
2/day	3.83	(2.08– 7.06)	<0.001*
3 or greater/day	14.86	(7.98 – 27.67)	<0.001*
Duration of fruit consumption on a daily basis			
<5 min	1		
5-10 min	1.90	(0.89 – 4.06)	0.097
>10 min	14.50	(6.43 – 32.70)	<0.001*
Duration of acidic drink consumption on a daily basis			
<5 min	1		
5-10 min	2.60	(1.24-5.44)	0.011*
>10 min	3.43	(1.85-6.36)	<0.001*
Drinks acidic drinks daily but does not sip/swish/hold drinks	1		
Drinks acidic drinks daily and sips/swishes/holds drinks	11.64	(5.49–24.67)	<0.001*

No statistically significant relationship was observed between brushing within 10 minutes of consuming a dietary acid and erosive tooth wear when dietary factors were fully adjusted for (OR 1.48, 95% CI: 0.87 – 2.51, p=0.147). The strongest relationship was observed when dietary acids were consumed between meals (≥ 3 intakes per day, OR 14.86, 95% CI: 7.98 – 27.67, p<0.001) although a positive linear relationship was observed between increasing daily frequency of dietary acid consumption and erosive wear both with meals and between meals.

Interestingly, a strong relationship was observed when greater than 10 minutes was taken to consume fruit daily (OR 14.50, 95% CI 6.43 – 32.70, $p<0.001$).

Alternative drinking methods such as sipping, swishing or holding the drinks in the mouth were also strongly associated with erosive wear (OR 11.64, 95% CI 5.49 – 24.67, $p<0.001$).

Although the same patterns remained when fruit and acidic drinks were analysed separately, no statistically significant relationship was observed between fruit intake with a meal and erosive wear. Daily consumption of fruit with two meals approached statistical significance (OR 1.99, 95% CI: 0.92-4.32, $p=0.083$). Acidic drinks, both with and between meals were associated with erosive wear although the strength of this relationship increased with daily acidic drink consumption between meals. Twice daily consumption of acidic drinks with meals and between meals was associated with odds ratios of 6.42 and 11.84 respectively.

Figure 34: Fruit and acidic drink intake analysed separately in a multivariate analysis

Variable	OR	95% CI	p value
Gender			
Male	1		
Female	0.37	(0.22 – 0.59)	<0.001*
Does the patient brush within 10 min of consuming a dietary acid?			
No	1		
Yes	1.41	(0.82 – 2.42)	0.215
Fruits with a meal			
Less than once daily	1		
1/day	1.36	(0.75 - 2.45)	0.316
2 or greater/day	1.99	(0.92 - 4.32)	0.083
Fruits between meals			
Less than once daily	1		
1/day	1.95	(1.02– 3.75)	0.017*
2 or greater/day	5.35	(2.51 – 11.43)	<0.001*
Acidic drinks with a meal			
Less than once daily	1		
1/day	1.81	(0.97 – 3.37)	0.061
2 or greater/day	6.42	(2.97 - 13.91)	<0.001*
Acidic drinks between meals			
Less than once daily	1		
1/day	2.49	(1.61-7.11)	0.010
2 or greater/day	11.84	(5.42-25.89)	<0.001*
Duration of fruit consumption on a daily basis			
<5 min	1		
5-10 min	2.47	(1.14 - 5.32)	0.022*
>10 min	12.82	(5.85 – 28.08)	<0.001*
Duration of acidic drink consumption on a daily basis			
<5 min	1		
5-10 min	2.35	(1.14-4.81)	0.020*
>10 min	3.08	(1.63-5.29)	<0.001*
Drinks acidic drinks daily but does not sip/swish/hold drinks	1		
Drinks acidic drinks daily and sips/swishes/holds drinks	10.34	(4.85–22.06)	<0.001*

4.6 DISCUSSION

This study confirms that the overall frequency of dietary acid consumption is associated with erosive wear. Interesting patterns emerge when dietary acid intake is separated by timing in relation to meals. Although the consumption of dietary acids both, with and between meals, were independently associated with erosive wear, the strength of association increased when dietary acids were consumed between meals. When acidic drinks and fruit were analysed separately (Figure 34), consumption of two or greater acidic drinks daily between meals carried the strongest relationship with erosive wear (OR 11.84). The odds ratios were almost halved when the same frequency of acidic drinks were consumed with meals (OR 6.82). Fruit intake with meals was not statistically associated with erosive wear, similar levels of fruit intake between meals were. Although the potential buffering capacity of meals has been discussed in previous studies, this is the first study to demonstrate clearly the protective effect of consuming dietary acids with meals. This discovery, may explain conflicting epidemiological studies, some of which have observed increased risk with dietary acids (Lussi and Schaffner 2000; Bartlett *et al.* 2013) and others which have not (Correr *et al.* 2009).

An overall linear relationship was observed between increasing frequency of dietary acid intake and erosive wear. However, odds ratios increased substantially at a certain level of intake. In this study, the odds of erosive tooth wear increased substantially when dietary acids were consumed three times daily between meals (Table 11, OR 14.86). However, when assessing fruit intake or acidic drink intake

in isolation, odds ratios increased at twice daily consumption. Other authors have reported similar figures in case-control studies performed in children (O'Sullivan and Curzon 2000; Lussi and Hellwig 2014) and adults (Järvinen *et al.* 1991; Lussi and Schaffner 2000). Less than daily consumption of dietary acids was not associated with an increased risk of severe erosive tooth wear in this study. Caution should be exercised when interpreting this as risk will be dependent on the timing of the acid intake, the erosive potential of the acids and salivary factors. However, it may serve as a clinical indicator when assessing risk potential.

Acidic drink intake has a stronger association with erosive wear than fruit intake and this supports the findings of other clinical studies (Bardolia *et al.* 2010; Bartlett, Fares, *et al.* 2011; Hasselkvist *et al.* 2014). However, a novel and interesting finding is the comparable risk when fruit is consumed over a prolonged period ≥ 10 minutes. Few participants (12% of the study population) spent ≥ 10 minutes consuming fruit at a single sitting. However, this characteristic was a highly significant predictor of erosive tooth wear (OR 12.82, 95% CI 5.85 – 28.08, $p < 0.001$). It is interesting that consuming acidic drinks over a period ≥ 10 minutes did not represent the same level of risk (OR 2.93). The physical act of chewing the fruit prior to swallowing may increase the force at which the acid is directed at the teeth disrupting the Nernst layer. It may also be due to differing buffering capacities between fruit and drinks and further research could be done in this area.

In addition, when acidic drinks were sipped, swished or held in the mouth prior to swallowing an increased OR of 10.34 (95% CI: 4.85 – 22.06, $p < 0.001$) was observed. This has been reported in other studies (O'Sullivan and Curzon 2000; Bartlett, Fares, *et al.* 2011; Muller-Bolla *et al.* 2015; Hasselkvist *et al.* 2016) and

reflects the importance of the increased contact time with the acid and the dynamic interaction between the acid and the dental surface.

Theoretically the container or holder of an acidic drink may affect the dynamics of the erosive challenge (Johansson *et al.* 2004) and a weak relationship between erosive wear and drinking from a bottle daily was observed in this study (OR 2.13, 95% CI 1.23-3.68, $p=0.007$). This lost significance when frequency of acidic drink intake was controlled for and was not included in the final multivariate analysis. It may be that drinking from a bottle may increase frequency of acidic drink consumption as it can be stored for later use. Very few other studies have investigated this. Moazzez *et al.* found that erosive wear patients were more likely to drink from a can although the sample size was small ($n=21$) (Moazzez *et al.* 2000). O'Sullivan questioned participants on the holder they frequently used but did not report on the findings (O'Sullivan and Curzon 2000).

A statistically greater number of erosive wear patients brushed their teeth within 10 minutes of consuming something acidic (OR 2.2, 95% CI 1.46-3.17, $p<0.001$), but this was not statistically significant when dietary risk factors were fully adjusted for (OR 1.41, 95% CI 0.82 – 2.42, $p=0.215$). Interestingly, a small protective association was observed (OR 0.55, 95% CI 0.35 – 0.86), $p=0.009$ when greater than 10 minutes elapsed before brushing teeth after breakfast. Further statistically insignificant but protective associations were observed when brushing was performed at mealtimes provided it did not occur within 10 minutes of the erosive challenge. Odds ratios decreased when brushing was performed after dinner and increased when brushing was not performed at breakfast time. This

may be suggestive that brushing with a fluoride dentifrice may play a protective role in erosive tooth wear (Ganss, Schlueter, *et al.* 2007; Wiegand *et al.* 2008).

Overall large differences were not detected between the two groups when assessing their daily tooth brushing behaviours outside of dietary acid intakes. Statistical differences were noted but the sample sizes were small. Although few patients brushed their teeth after lunch, a statistically greater number of them were erosive wear patients (OR 2.73 [95% CI 1.32 – 5.62], $p=0.007$). This may reflect that acidic items are being consumed at lunchtimes as this study found no association between the number of times teeth were brushed daily and erosive wear. Other studies have also observed no relationship between frequency of brushing and erosive wear (Dugmore and Rock 2004a; Bartlett *et al.* 2013), with others reporting a positive association (Bader *et al.* 1996; Lussi and Schaffner 2000) and one other reporting the opposite; decreased frequency of tooth brushing was associated with increased wear (Hasselkvist *et al.* 2014). There is a possibility that conflicting results in epidemiological studies may be as a result of the timing of tooth brushing in relation to a dietary acid. Unfortunately, further longitudinal with large sample sizes would be needed to confirm this.

This study also observed that more erosive wear patients reported using a hard manual toothbrush (OR 3.19, 95% CI: 1.44 – 7.06, $p=0.004$). This result agrees with the results of a longitudinal epidemiological study reporting that the use of a hard toothbrush was associated with increased wear progression (Lussi and Schaffner 2000) but conflicts with recent laboratory data observing that soft toothbrushes may result in increased abrasive wear (Tellefsen *et al.* 2011; Bizhang *et al.* 2016). The self-reported use of a hard toothbrush may be a reflection of an aggressive

tooth brushing style which has been observed to result in increased wear (Bartlett *et al.* 2013). The results of this study may indicate that prevention advice should be focused on the diet.

It was felt that age was important to control for as biological wear increases as the dentition ages. Frequency matching was successfully employed within this study with no significant differences observed between the groups with respect to age. More males presented with erosive wear than females ($p=0.003$), which was adjusted for statistically in all analysis. The increased association between the male gender and erosive wear has been observed in other large epidemiological studies (Al-Dlaigan *et al.* 2001; Bardsley *et al.* 2004; Dugmore and Rock 2004b; El Aidi *et al.* 2008; Mulic *et al.* 2012; Alvarez Loureiro *et al.* 2015; Okunseri *et al.* 2015), with others observing no difference (Peres *et al.* 2005; Auad *et al.* 2007; Bartlett *et al.* 2013) and others showing a higher prevalence in females (Wang *et al.* 2010; Huew *et al.* 2012). It is relatively unknown whether this difference is behavioural or physiological. There are no clear trends with regards to salivary differences (Schlueter and Tveit 2014). Bardsley *et al.* 2004 suggested that it may be due to higher occlusal forces generated by males (Bardsley *et al.* 2004) whereas others have hypothesised that differing patterns of dietary consumption may play a role (Mulic *et al.* 2012). It is recognised that males are more likely to suffer from gastro-oesophageal reflux disease (Schlueter and Tveit 2014), which may be asymptomatic and a potential explanatory factor. Longitudinal studies may be needed to determine if greater incidences of erosive wear within genders is due to behavioural or physiological mechanisms.

Erosive wear patients were more likely to report they were currently suffering with dental hypersensitivity ($p < 0.001$). However, a large proportion of controls also reported to be currently suffering with dental hypersensitivity (41.7%). Studies suggest the prevalence of dental hypersensitivity to be between 7.6-68.4% (West *et al.* 2013) and we are aware that dietary acids have the potential to open tubules causing sensitivity (West *et al.* 2013; Olley *et al.* 2015). The large proportion of dental hypersensitivity observed in controls may be that those attending a hospital setting for dental care may have a higher level of sensitivity than the general population. It is also unknown the threshold at which dietary acids cause hypersensitivity. It could be that a low level of dietary acid consumption may be sufficient to cause hypersensitivity but not tooth wear in susceptible individuals. Further research is needed in this area investigating the relationship between acid exposure and dental hypersensitivity.

This study was based on hospital volunteers, which may limit the ability to generalise findings beyond the study population. Furthermore, dietary assessment in this study was based on current patterns of consumption. Erosive damage to the dentition may have occurred at any stage post eruption of the permanent dentition where a different diet was consumed. This is a limitation of retrospective questionnaire-based studies where existing and new incident cases are difficult to identify. It is recommended that future research focuses on longitudinal studies with multiple dietary assessments over time.

Interviewer-led questionnaires are also subject to reporting bias. There is the possibility that patients who were diagnosed with erosive wear were more aware of dietary acid intake which could have resulted in under or over reporting of risk

factors. The clinical setting may have resulted in social desirability bias whereby participants reported answers which they perceived they would not be judged poorly on. Although attempts were made to minimise these biases by maintaining neutral wording, offering no feedback on answers and questioning erosive wear patients and controls in the same manner, it is difficult to gauge the full effect of these biases.

Salivary analysis was not performed in this study as it was not the aim of the research. This may be a further explanatory factor in the variation between participants in diet and erosion.

Control of other confounding factors such as parafunctional habits, gastric symptoms, eating disorders and xerostomic drugs was attempted by excluding all diagnosed cases. These conditions are reliant on a diagnosed condition recorded in the medical notes or self-reporting via the patient and it is possible that cases were included with intrinsic erosion as the primary cause of wear. Given the large sample size within this study it is estimated that the inclusion of these rare cases would not impact the results significantly.

Finally, epidemiological data suggest that the population of many countries do not have adequate fresh fruit consumption (Health and Social Care Information Centre 2015). Consideration should be given to the overall health of the individual when providing dietary advice.

4.7 CONCLUSION

Erosive tooth wear was observed to be statistically significantly associated with the frequency of dietary acid intake and duration of dietary acid consumption in this study. Therefore the first two null hypotheses were rejected. The predominant

risk factors in the development of severe erosive tooth wear in this study were frequency of acid consumption between meals, eating fruit over an extended time period and an alternative drinking method such as sipping, swishing or holding acidic drinks in the mouth prior to swallowing. Brushing after meals was not associated with erosive tooth wear when dietary factors were adjusted for. This supported the third null hypothesis and may suggest universal preventive advice to delay brushing after meals is not substantiated. However, there is insufficient evidence to fully exclude brushing within 10 minutes of acid intake outside of meals as a risk factor based upon the results of this study. These findings help characterise dietary patterns more strongly associated with tooth wear. Prospective, longitudinal studies incorporating multiple dietary assessments are recommended to confirm the results of this study.

CHAPTER 5: PROSPECTIVE RANDOMISED CONTROLLED CLINICAL TRIAL INVESTIGATING DIETARY ADVICE AS AN INTERVENTION IN TOOTH WEAR PROGRESSION

5.1 OVERVIEW

The previous chapter reported that the frequency of dietary acid intake was associated with erosive tooth wear, particularly when dietary acids were consumed between meals. However, it is unknown if reducing the frequency of acid intake between meals prevents tooth wear progression.

To date no studies have shown provision of dietary advice to have an impact on the progression of tooth wear (Lussi and Schaffner 2000; Harris *et al.* 2012).

Furthermore, there is limited evidence suggesting dietary advice can result in behavioural change (Harris *et al.* 2012). The use of applied psychology theory to induce a behaviour change has been successfully utilised when applied within a clinical setting (Renz *et al.* 2007; Suresh *et al.* 2012; Michie *et al.* 2015) and may be promising when applied to erosive tooth wear. Ideally, an objective clinical outcome to measure adherence to the behaviour change should be utilised when assessing the effectiveness of a technique employed (Harris *et al.* 2012; Adair *et al.* 2013).

The digitisation of study models and surface matching software have been used by different research groups when attempting to monitor tooth wear (Lambrechts *et al.* 1989; Pintado *et al.* 1997; Chadwick *et al.* 2005; Rodriguez *et al.* 2012a; Ahmed *et al.* 2015). Previous methods used include volume change (Pintado *et al.* 1997; Tantbirojn *et al.* 2012), profilometric loss (Chadwick *et al.* 2005; Rodriguez *et al.* 2012b) and maximum single point loss (Lambrechts *et al.* 1989).

The aim of this study was to assess the impact of dietary advice on the rate of tooth wear progression over a 6-month period. A further aim was to compare different methods of tooth wear assessment using surface matching software.

5.2 NULL HYPOTHESIS

1. A behaviour change intervention will not change dietary acid intake compared to standard of care dietary advice.
2. A behaviour change intervention will not impact tooth brushing behaviours compared to standard of care dietary advice.
3. A behaviour change intervention will not change the progression of erosive tooth wear.

5.3 MATERIALS AND METHODS

This study was a double blind, randomised, controlled, parallel group, clinical trial.

The study protocol was approved by Nottingham National Health Research Authority East Midlands and all participants provided informed written consent (Reference 14/EM/1171). This study adheres to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Schulz *et al.* 2010) and was registered under clinicaltrials.gov.uk (registration ID: NCT02493803). Partial funding was received by Proctor and Gamble in the form of a PhD studentship.

5.3.1 DEVELOPMENT OF THE DIETARY ADVICE INTERVENTION

For the intervention group, an If-then plan was developed with dental psychologist Professor Timothy Newton using the COM-B model (Capability, Opportunity, Motivation – Behaviour change (Asimakopoulou and Newton 2015)). This plan aimed to provide knowledge (targeting capability) and link this with specific

environmental contexts (targeting opportunity). A prompt-sheet with a list of dietary acids and healthy substitutions with low erosive potential was formulated alongside senior dietician Dr Jane Thomas (Appendix 8.6). For each behaviour requiring change, participants would be asked to identify and write down the behaviour they wished to change and the substitution/abstention they were going to make. Participants would then be prompted to identify obstacles that would prevent them from making their chosen substitution/abstention (e.g. partner drinks the acidic drink as well or the participant has a habit of buying acidic fruit). Participants would then be prompted to consider how they might overcome these obstacles (e.g. is there anything else that the partner and participant could consume together? Is there another snack that could replace the acidic fruit between meals?). Finally, participants would then be asked to consider and write down items that would help them to make the change (e.g. asking the partner to help them make the change, buying different types of drinks/snacks that were equally appealing). Participants would then be encouraged to place the plan in a place that would be visible daily at work or at home (Appendix 8.7).

The intervention was designed to target two behaviours: the frequency of dietary acid consumption between meals and brushing teeth within 10 minutes of consuming a dietary acid. Researcher scripts and guidelines were formulated to ensure dietary interventions were standardised and are presented in Appendix 8.8.

The standard of care diet advice given to the control group would consist of the statement: *"Our examination has revealed that you show signs of erosive damage on your teeth. This is most likely to be due to a combination of the foods and drinks that*

you choose, when you have them and when you brush your teeth. We recommend that you cut down on the frequency of having acidic foods and drink.”

Both forms of diet advice were piloted on a group of 10 volunteers to ensure legibility, comprehension and researcher standardisation.

5.3.2 PARTICIPANTS

Previous work within our group observed differences of 15 μm between participants with high and low levels of wear progression (Rodriguez *et al.* 2012a), which would yield an effect size of 0.78. In order to detect a difference in wear with 80% power and at the 5% level of significance and effect size of 0.78, a total sample size of 54 participants (27 participants in each group) is required. We recruited 60 participants anticipating a 10% dropout rate.

The source population were participants either referred via their general dental practitioner or self-referred for assessment at consultant or generalised restorative clinics in King's College London Dental Institute, Guy's Hospital between December 2014 and February 2016. Participants of both sexes, aged between 25 and 70 years, identified by their examining dentist as having at least one sextant affected by severe erosive tooth wear (BEWE = 3) were approached and invited to take part in a screening examination assessing eligibility to participate. The full participant information sheet was explained to the patient and, following verbal consent, a screening examination was performed.

The medical history was checked and the Basic Erosive Wear Examination (BEWE) index used to assess erosive tooth wear using methods identified in Chapter 3, section 3.2.1. Examinations were carried out in a dental chair with the patient in a reclined position and good lighting. The teeth were dried and cleaned with

compressed air and the buccal, occlusal and palatal/lingual surfaces of each tooth excluding third molars were each examined without magnification. The highest score in each sextant was recorded. These were then summed to give a total BEWE score (Bartlett *et al.* 2008).

Diet was assessed and risk factors identified using the questionnaire developed and validated in Chapters 3 and 4 (Appendix Section 8.2). Participants were questioned on the frequency and timing of dietary acid intake, the time spent consuming acids and alternative drinking habits prior to swallowing. In addition, they were questioned on the timing of their tooth brushing in relation to meals and dietary acid intake. The dietary inclusion criterion for this study was consumption of two or more dietary acid intakes per day. The inclusion criteria (Appendix Section 8.4) were: a minimum of 20 teeth (10 in each jaw) with a BEWE cumulative score greater than or equal to 8 but with at least one score of 3 on the occlusal surfaces of the lower molars or the buccal/palatal surface of the upper central incisor, the cause of the wear was a high acid diet (at least two daily incidences of dietary acid intake) and participants were able to provide written consent to the study. Participants were excluded if they had orthodontic appliances, severe dental hypersensitivity, missing anterior teeth, anterior crowns/bridges or cavitated caries on more than one tooth. A history of eating disorders, gastro-oesophageal reflux, xerostomia, bruxism, prescribed xerostomic/heartburn medication, pregnancy, involvement in other research within the past 30 days or inability to speak or understand the English language also excluded the participant from this study. Those with medical histories likely to impact on compliance (e.g. requiring

antibiotic pre-medication prior to dental treatment) or those preferring immediate restoration of their teeth were also excluded.

5.3.3 DATA COLLECTION

Participants were given an opportunity to ask further questions and given a minimum of 24 hours to consent. Following consent, a separate appointment was given (T0) and patients allocated a unique trial identifier number based upon sequence of recruitment. Simple Random Sampling (SRS), using Excel (Microsoft Office Excel 2010, Redmond, USA), was used to allocate the patient to either the intervention or standard care group. The participant was blinded to which dietary intervention s/he received. The blinding process for the clinical investigator occurred during analysis.

If assigned to the intervention group, two risk factors were targeted using the methodology described in Section 5.3.1. If the patient consumed dietary acids between meals, the implementation plan was targeted to reduce the consumption between meals. If the patient brushed her/his teeth within 10 minutes of consuming a dietary acid, the implementation plan was targeted to suggest s/he brush her/his teeth before the erosive challenge, i.e. before breakfast or lunch. All interventions were standardised using the intervention process described in Section 5.3.1. Each intervention lasted 3-5 minutes. For those not assigned to receive the intervention, participants were given the standardised dietary advice as noted in section 5.3.1. This intervention lasted less than 1 minute.

Following the intervention, dental impressions were taken. The patient was placed in a supine position in a dental chair. Under good lighting conditions, the teeth were dried and cleaned with compressed air. An appropriately sized stock tray was selected and an alginate impression taken of the upper and lower arches (Alginate Plus, DF Fast Set, Henry Schein, Kent, UK) to remove any debris present.

The index teeth were then isolated with cotton wool rolls and dried with compressed air. New stock trays were coated in a thin layer of polyvinyl siloxane adhesive (VPS Hydro Adhesive, Henry Schein, Kent, UK) and upper and lower addition-cured silicone impressions taken using gun-dispensed medium body base and separate light body wash (Extrude, Kerr Dental, Peterborough, UK). The impressions were checked for accuracy. If flaws were noted on the index teeth, the impression was discarded and repeated with fresh material. Accurate impressions were disinfected by immersing in Perform ID (Schülke & Mayr UK Ltd. Sheffield, UK) for 10 minutes according to manufacturer's instructions and left to rest undisturbed for 24 h before being poured in type 4 dental stone (Fujirock EP, premium line pastel yellow, GC United Kingdom Ltd., Newport Pagnell, UK). The dental stone was vacuum-mixed according to the recommended water/powder ratio at 25–30 mm Hg negative pressure.

Participants returned for the review visit (T1) 6 months (± 7 days) later when the dietary questionnaire, alginate and silicone impressions were repeated following the same process described above. Again, polyvinylsiloxane impressions were left to rest undisturbed for 24h before being cast in the same dental stone using the same process.

After a minimum of 24 hours, the previously air cleaned casts were scanned using a triangulation laser profilometer (Xyris 2000TL. TaiCaan, Southampton, UK). The buccal and palatal surfaces of the central incisors and the occlusal surfaces of the lower first molars were mapped using the laser profilometer. Data readings were taken every 50 μm , scanning from left to right in a raster pattern, at medium precision mode (scanning speed of 2.81 mm/s) using the same methods described

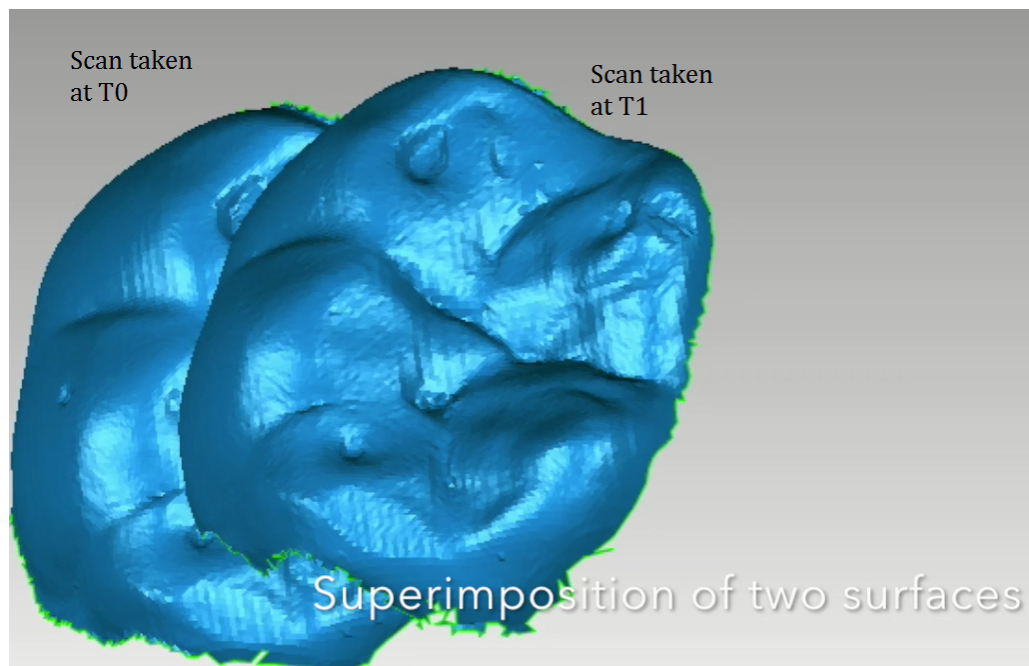
in Rodriguez *et al.* 2012. This generated an accurate 3D surface profile of the tooth surface. Total scanning time was 45-60 min per set of casts depending on the size of the surface to be scanned.

5.3.4 DATA ANALYSIS

Self-reported changes in frequency of dietary acid intake and tooth brushing behaviours were extracted from the diet questionnaires obtained at T0 and T1. The overall daily consumption of fruit and acidic drink intake was calculated in addition to the frequency of fruit and acidic drink intake between meals. The change was calculated by subtracting the number of times dietary acids were consumed at T1 from the number of times per day that dietary acids were consumed at T0. If the patient brushed her/his teeth within 10 minutes of consuming a dietary acid at T0, it was noted if s/he had stopped this behaviour at T1.

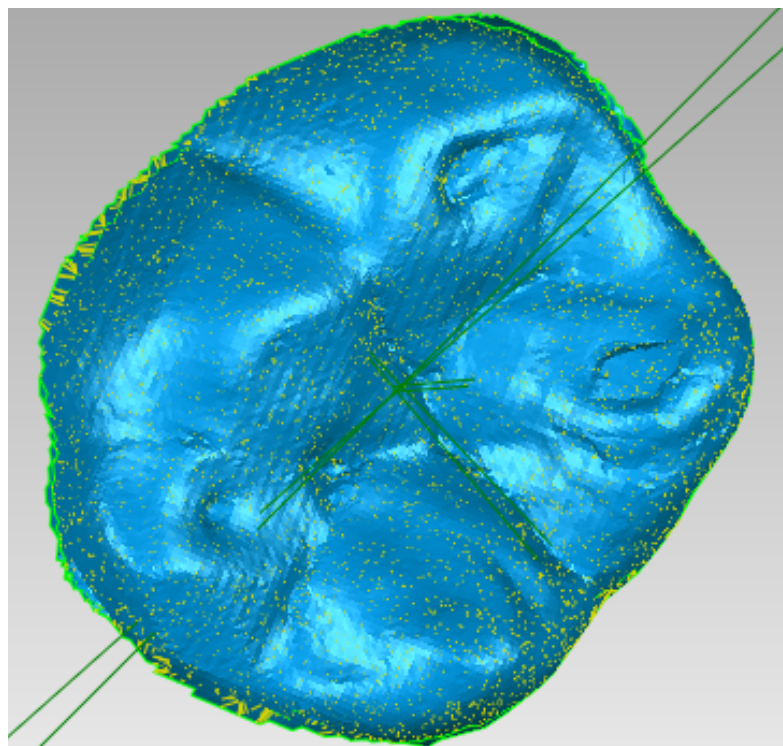
The 3D surface profiles obtained at T0 and T1 were superimposed using Geomagic Control software (3D systems, Darmstadt, Germany). This programme aligns two sequential scans (Figure 35) by comparing the root mean square difference between given numbers of data points for each scan.

Figure 35: Image demonstrating two separate scans of a lower left molar prior to alignment and trimming using Geomagic Control software



The number of data points chosen to align the scans determines the accuracy of the fit. These are represented by the small yellow dots in figure 36.

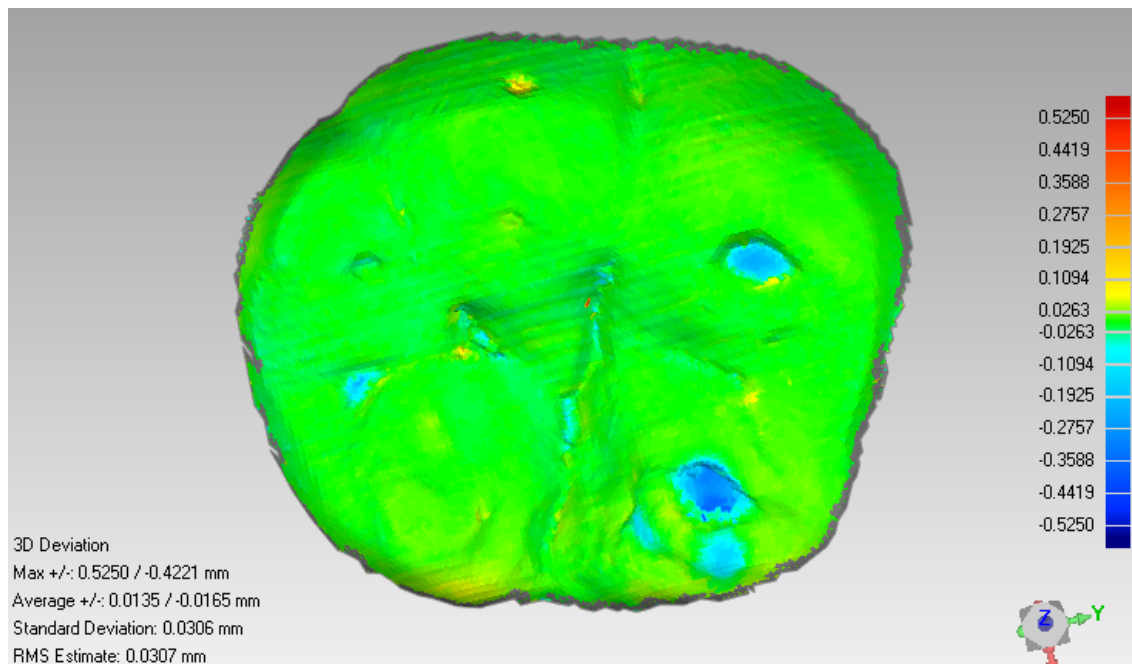
Figure 36: Geomagic software in the process of aligning two scans. Data points which the software is trying to minimise differences between are the small yellow dots on the surface



However, the accuracy of superimpositional fit was influenced by discrepancies in shape between the two scans, particularly those at the periphery. An example of this is shown in figure 35 where demarcation of the periphery is not clear. For this reason, a rough alignment was performed using 300 data points and following this the two scans were trimmed to ensure they were closer in shape, facilitating a more accurate superimpositional analysis. This process was repeated with a refined alignment using 5,000 data points, trimming the circumference of the scans until they were of equal size and shape. Once discrepancies in surface areas between the two scans were eliminated, a final precision alignment using 10,000 data points was performed.

At this stage a heat map of the surface depicting profilometric changes was generated to visualise wear patterns. An example of this is observed in figure 37 where the green areas represent little or no change, deepening areas of blue represent deepening areas of loss and yellows represent positive gain. However, despite these stages the area identified around the circumference of the scan was not consistent between T0 and T1. These inaccuracies overwhelmed the differences between the scans.

Figure 37: Colour representation of profilometric changes. The scale on the right is in mm



To standardise the area analysed between each surface, a 7 x 4 mm representative sample area was conveniently selected from the central part of the digitised surface. The centre was arbitrarily defined at the intersection of the mesio-distal and bucco-lingual length for molars as shown in Figure 39 and at the intersection of the cervico-incisal and mesio-distal length for incisors (Figure 40).

Figure 38: Standardised surface area analysed on the occlusal surface of a lower molar

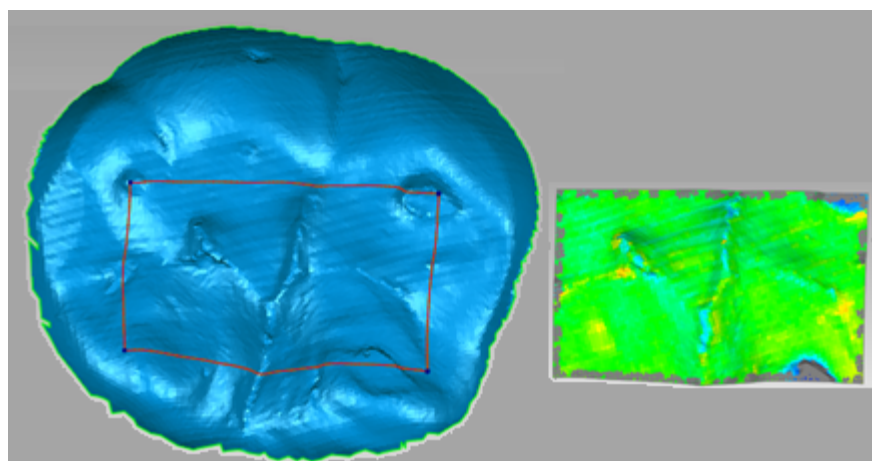
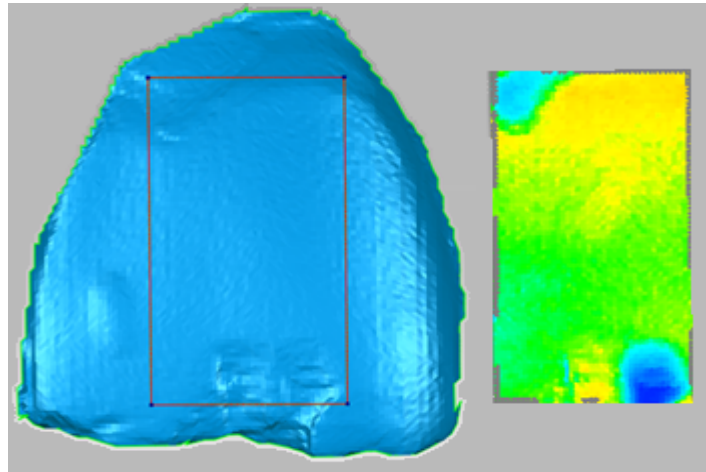
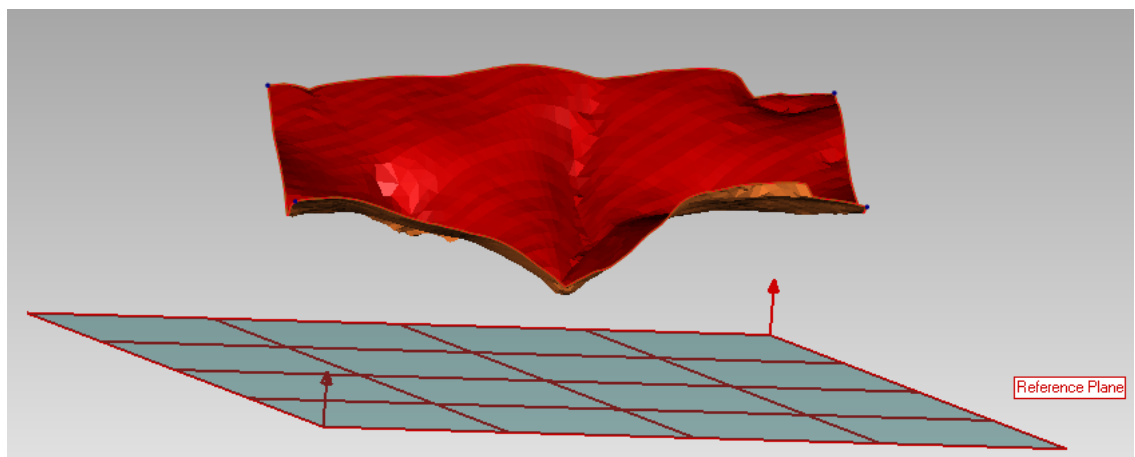


Figure 39: Standardised area analysed on the buccal surface of a central incisor



Once these areas were standardised and aligned, the volume and step height change was calculated by establishing a digital reference plane under both of the aligned surfaces as seen in Figure 41. The software calculated the volume between the plane and the scanned surface for both T0 and T1. The change was calculated by subtracting the data recorded at T0 from that taken at T1.

Figure 40: A reference plane was established which allowed the software to calculate the volume under the 3D scan



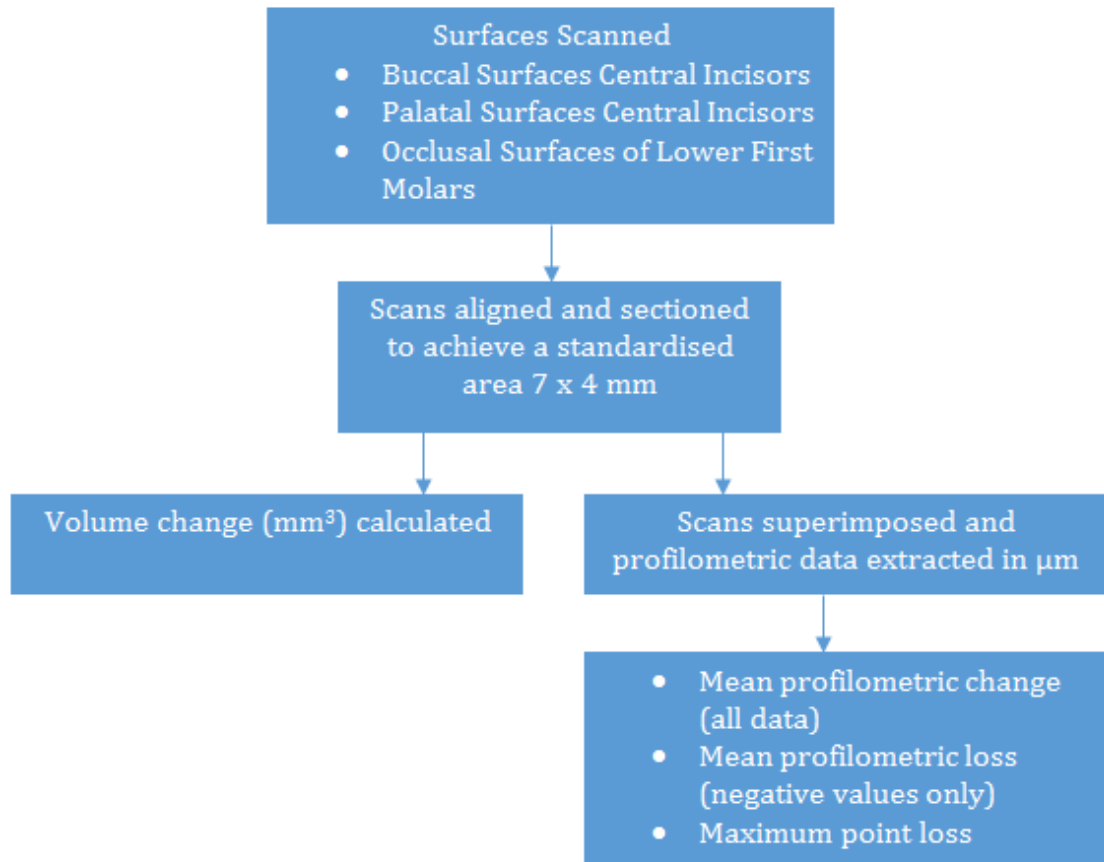
The software was also used to calculate height (profile) differences between each data point superimposed. In total four outcomes were generated. The volume represented the difference between T0 and T1 in mm³. The profilometric change was the mean of all data points both positive and negative values, whereas the

profilometric loss represented the average of only the negative profilometric height loss values. Maximum point loss was the largest negative height discrepancy between T0 and T1 (Figure 41).

Figure 41: Explanation of tooth wear measurements used

Volume change (mm³)	Volume of surface at visit 1 minus the volume of surface at visit 2.
Profilometric change (µm)	The mean of all data points deviations (positive and negative) measured on the Z axis (n=7,000-10,000 per scan depending on size of surface).
Profilometric loss (µm)	Mean value of negative data point deviations only measured on the Z axis (Rodriguez <i>et al</i> 2012).
Maximum point loss (µm)	Maximum loss detected within the analysed area.

Figure 42: Overview of erosive tooth wear progression measurements



Scanning, superimposition and data analysis were performed by the author who was blinded to the group during analysis.

Repeatability and reproducibility errors of the process were also assessed. To assess repeatability, a randomly selected surface on a randomly selected cast was scanned ten times; each time the cast was repositioned on the profilometer stage between scans. Using the first scan as a baseline, the following 9 scans were aligned and superimposed to assess the error.

To assess the reproducibility error, five maxillary impressions of a randomly selected volunteer were taken at the same appointment, poured up and scanned using the same method as described in section 5.3.3. The study cast obtained from

the first impression acted as the baseline, and subsequent scans from the other 4 casts were superimposed.

5.3.5 STATISTICS

All analyses were performed in the IBM SPSS Statistics 22 (IBM Corporation, Armonk, New York). Data were checked for normality using histograms, boxplots and the Shapiro-Wilk test. Data were not normally distributed and were assessed using Mann Whitney U tests and intra-group analysis was performed using the Wilcoxon Signed Rank test.

Data were analysed at patient level and at surface level with further analysis investigating differences between the surface type (buccal surfaces of central incisors, palatal surfaces of central incisors and occlusal surfaces of lower first molars). Differences in the reproducibility data between the surfaces were assessed using the Kruskal-Wallis test. Mann Whitney U tests were used to assess differences between groups. Bivariate relationships were assessed using Spearman's Rank Correlations. Interpretation of Spearman's correlations were used according to Hinkle *et al.* 2003 whereby 0-0.3 indicated a negligible correlation, 0.3-0.5 a low correlation, 0.5 to 0.7 a moderate correlation and above 0.7 to be a high correlation. Significance was inferred at $p < 0.05$.

5.4 RESULTS

5.4.1 PATIENT DEMOGRAPHICS

Of the total 98 participants assessed for eligibility, 33 did not meet the inclusion criteria and 5 declined to participate. An initial sample size of 60 was randomly assigned to each intervention and 30 were assigned to each group. One participant from each group was lost to follow-up as s/he was not in the country at the time of recall. In addition, one participant from the control group was lost to a fatal accident not related to the trial. This resulted in a total of 28 participants in the control group and 29 participants in the intervention group. The mean age for the control group was 37.7 years (SD = 11.7, Range 25-61) and 36.5 years (SD = 11, Range 25-69) for the intervention group. More females were in the control group (n = 17, 60.7%) compared to the intervention group (n=12, 41.4%). The baseline mean total BEWE score was 14.7 (SD =2.5) for the control group and 14.8 (SD = 2.2) for the intervention group. Figure 43 shows the distribution between the two groups with no statistical differences observed in age (p=0.529), sex (p=0.116), or baseline total BEWE score (p=0.769). No statistical change was observed in clinical BEWE scores over the 6-month observation period (p=0.849).

Figure 43: Demographics

		Control Group (n=28)		Intervention Group (n=29)		p value
		n	%	n	%	0.529
Age						
	18-25	4	14.3	4	13.8	
	26-35	11	39.3	13	44.8	
	36-45	5	17.9	5	17.2	
	46-55	4	14.3	6	20.7	
	56-65	4	14.3	1	3.4	
Gender						0.116
	Male	11	39.3	17	58.6	
	Female	17	60.7	12	41.4	
		Mean	SD	Mean	SD	0.769
BEWE		14.7	(2.5)	14.8	(2.2)	

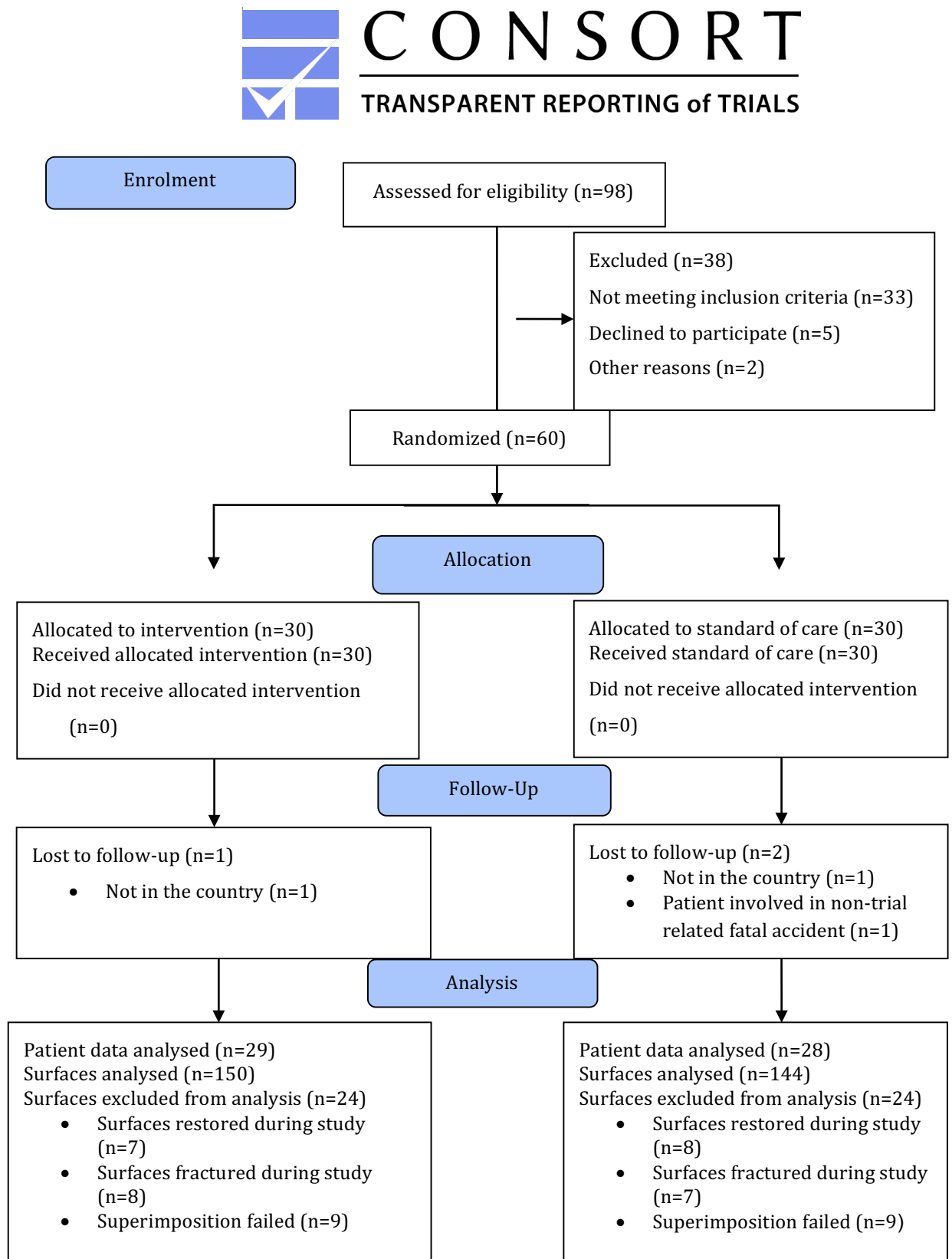
5.4.2 OVERVIEW OF DATA COLLECTION

All participants who completed the trial (n=57), successfully completed both diet questionnaires and had two sets of dental silicone impressions. The quality of impressions on the index teeth was acceptable and no impressions were excluded. Each impression was poured in type 4 stone with no defects noted on the index teeth. The quality of the impressions and stone casts were visually assessed and checked with a travelling microscope. The occlusal surfaces of the lower first molars and the palatal and buccal surfaces of the central incisor were scanned from a total of 228 casts (4 casts per patient) at T0 and T1. This resulted in 684 surfaces or 342 surface pairs. Each scan took 10-12 minutes depending on the scan.

For the intervention group (n=29) a total of 150 surfaces were analysed from a possible 174. The reasons for exclusion were restoration of the surface (n=7), fracture of the surface (n=8) and failure of accurate data point alignment (n=9). For the control group (n=28), a total of 144 surfaces were analysed out of a possible 168. The reasons for exclusion were restoration of the surface (n=8), fracture of the surface (n=7) and failure of accurate data point alignment (n=9).

A flow chart for the data collection for the intervention and control group is reported in Figure 44 according to Consolidated Standards of Reporting Trials (CONSORT) guidelines (Schulz *et al.* 2010).

Figure 44: CONSORT flow diagram for reporting trials



5.4.3 SELF-REPORTED BEHAVIOUR CHANGES

Frequency of total daily dietary acid intake.

At T0, the control group consumed dietary acids a median frequency of 4 times per day (IQR 2, 5.8, Range: 2 - 9 per day). The intervention group consumed dietary acids a median of 4 times per day at T0 (IQR 2, 5, Range: 2 - 6) with no statistically significant ($p=0.633$) differences between groups at baseline. At T1, a median of 2.5 intakes for the control group (IQR 1, 4, Range: 0 - 6) and 1 intake for the intervention group (IQR 0.5, 2, Range: 0 - 4) was recorded. The median reduction for the control group was 1 intake (IQR 0, 2.75, Range -1 - 9), $p=0.001$ and 2 intakes for the intervention group (IQR 1, 3.5, Range -2 - 6), $p<0.001$. However, differences between groups were not statistically significant, $p=0.078$.

Frequency of daily dietary acid intake between meals.

At T0, the control group consumed dietary acids between meals a median frequency of 3 times per day (IQR 2, 4, Range: 0 - 6) and the intervention group a median of 3 times daily at baseline (IQR 2, 4, Range 0 - 8) and this difference was not statistically different ($p=0.783$). At T1, both the control group and intervention group reduced their frequency of dietary acid intake to a median of 1 intake between meals (control group: IQR 1, 3, Range 0- 4, and the intervention group: IQR 0, 3, Range 0 - 4). However, the overall median change was 1 for the control group (IQR 0, 3, Range: -1 - 6) and 3 for the intervention group (IQR 1, 3, Range -1 - 7) and this was statistically significantly different ($p<0.001$).

Ten participants in the control group and eleven participants in the intervention group reported to brush their teeth within 10 minutes of consuming a dietary acid at T0. Of those, four participants (40%) in the control group did not report this

behaviour at T1, compared to seven (63.6%) participants in the intervention group. Although a greater number of participants in the intervention group stopped brushing within 10 minutes of dietary acid intake, this was not statistically significant ($p=0.387$). Behaviours at baseline, after the trial and changes are reported in Figure 45.

Figure 45: Self-reported behaviour changes

	Control Group		Intervention Group		p value
	Median	IQR	Median	IQR	
Total daily acid intake T0	4	(2, 5.8)	4	(2, 5)	
Total daily acid intake T1	2.5	(1, 4)	1	(0.5, 2)	
Median change between T0 and T1	1	(0, 2.8)	2	(1, 3.5)	0.074
Acid intake between meals T0	3	(2, 4)	3	(2, 4)	
Acid intake between meals T1	1	(1, 3)	1	(0, 1)	
Median change between T0 and T1	1	(0, 3)	3	(1, 3)	0.048*
Participants brushing within 10 minutes of consuming an acid at baseline	10	36%	11	38%	0.387
Stopped brushing after acid	4	40%	7	63.6%	
No change	6	60%	4	36.4%	

5.4.4 REPEATABILITY AND REPRODUCIBILITY OF DIFFERENT METHODS OF TOOTH

WEAR MEASUREMENT

Figure 46 shows the repeatability and reproducibility error measurements for all surfaces were analysed.

For the repeatability, the median volume change error was an overall gain of 0.01mm^3 (IQR 0, 0.2). The median profilometric change error was $0\text{ }\mu\text{m}$ (IQR -0.1,

0.3). The median profilometric loss error was 2 μm (IQR 1, 3.5) and the median maximum single point loss error of 21 μm (IQR 18, 52).

For the reproducibility, the median volume change error was 0.005mm³ (IQR -0.09, 0.03)), the median profilometric change error was 0.2 μm (IQR -0.4, 1.8), the median profilometric loss error was 11 μm (IQR 9, 18) and the median maximum single point loss error was 86.5 μm (IQR 78, 273).

Figure 46: Repeatability and reproducibility for all surfaces

	Repeatability *		Reproducibility **	
	(n=9)		(n=24)	
	Median	IQR	Median	IQR
Volume change (mm³)	0.01	(0, 0.02)	0.005	(-0.09, 0.03)
Profilometric change (μm)	0	(-0.1, 0.3)	0.2	(-0.4, 1.8)
Profilometric loss (μm)	2	(1, 3.5)	11	(9, 18)
Maximum point loss (μm)	21	(18, 52)	86.5	(78, 273)

*Same surface, same cast, scanned 10 separate times and superimposed 9 separate times using first scan as baseline. Theoretically all values should be zero. This represents scanning and superimposition error.

**Same surfaces from 5 different impressions/casts of the same person taken one after the other and superimposed. Theoretically all values should be zero. This represents the total impression taking, casting, scanning and superimposition error.

The reproducibility error data were then analysed further by surface type. The measurement error increased when analysing the molar surfaces compared to incisor surfaces (Figure 47). The median volume change for the buccal surfaces and palatal surfaces of the central incisors were positive, i.e. error showed an overall gain in volume (0.005mm³ (IQR -0.03, 0.18) and 0.0025mm³ (IQR -0.14, 0.06), respectively). In contrast, volume change for the occlusal molar surfaces was negative (-0.02mm³ (IQR -0.12, 0.03)).

The profilometric change error observed for the buccal incisor surfaces was 0.1 µm (IQR 0.0.3) and palatal incisor surfaces was 2.6 µm, (IQR 0.1, 3.3). Conversely, a negative profilometric change was observed for the occlusal molar surfaces -0.8 µm, (IQR -3.8, 1.2).

The median profilometric loss was 9 µm both for the buccal (IQR 8, 12) and the palatal incisors (IQR 8, 11). Median profilometric error for the occlusal surfaces of the upper first molars was 22.5 µm (IQR 18, 28); a significant increase in error compared to the incisal surfaces p<0.001.

The maximum point loss errors for the buccal and palatal surfaces of the central incisors were 83 µm (IQR 75, 91) and 80 µm (IQR 70, 84) respectively. Median maximum point loss error for the occlusal surfaces of the upper first molars was 312 µm (IQR 266, 368). This again was a significant increase in error compared to the incisal surfaces p=0.038.

Figure 47: Reproducibility data analysed further by surface type

	Buccal Incisors (n=8)		Palatal Incisors (n=8)		Upper Molars (n=8)		p value
	Median	IQR	Median	IQR	Median	IQR	
Volume change (mm³)	0.005	(-0.03, 0.18)	0.025	(-0.14, 0.06)	-0.02	(-0.12, 0.03)	0.727
Total profilometric change (µm)	0.1	(0.0, 0.3)	2.6	(0.1, 3.3)	-0.8	(-3.8, 1.2)	<0.001
Profilometric loss (µm)	9	(8, 12)	9	(8, 11)	22.5	(18, 28)	<0.001
Maximum point loss (µm)	83	(75, 91)	80	(70, 84)	312	(266, 368)	0.038

5.4.5 CORRELATION BETWEEN EROSIVE TOOTH WEAR MEASUREMENTS

Correlations between erosive tooth wear measurements for all surfaces were analysed (n=294). Two moderate correlations were noted between different methods of tooth wear measurements. Volume change was negatively correlated with profilometric change ($r = -0.67$, $p < 0.01$) and profilometric loss was positively correlated with the maximum point loss ($r = 0.62$, $p < 0.01$). No correlations were observed between volume change and profilometric loss. All correlations between different methods of tooth wear measurements are reported in figure 48.

Figure 48: Correlation matrix between different parameters when measuring all surfaces

	Volume change	Profilometric change	Profilometric loss	Maximum point loss
Volume change	1	-.67**	.08	.14*
Profilometric change	-.67**	1	-.16**	-.12*
Profilometric loss	.08	-.16**	1	.62**
Maximum point loss	.14*	-.12*	.62**	1

*Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Correlations between tooth wear measurements on the buccal surfaces of the central incisors were higher than correlations between measurements on the other surfaces. Volume change was weakly negatively correlated with the maximum point loss ($r = -0.26$, $p < 0.01$), and highly correlated with the profilometric change ($r = -0.7$, $p < 0.01$). The correlation between the volume change and profilometric change remained for the palatal surfaces of the central incisors ($r = -0.50$, $p < 0.01$) and the occlusal surfaces of the lower first molars ($r = -0.73$, $p < 0.01$). Profilometric

loss was also consistently correlated with the maximum point loss for the buccal ($r = 0.49, p < 0.01$) and palatal ($r = 0.63, p < 0.01$) surfaces of the central incisors and the occlusal surfaces of the lower first molars ($r = 0.47, p < 0.01$). Correlation tables between the different methods of tooth wear measurement for the buccal/palatal and occlusal surfaces can be seen in Appendix 8.9.

5.4.6 QUANTITATIVE EROSIVE WEAR MEASUREMENTS

5.4.6.1 Volume change

At the patient level, the data for the control group were observed to have a median volume change of -0.09 mm^3 (IQR $-0.14, 0.05$) per surface and for the intervention group a median volume change of -0.02 mm^3 (IQR $-0.08, 0.09$) per surface and this was not statistically significant, $p=0.078$.

At surface level, the control group had a median volume change per surface of -0.06 mm^3 (IQR $-0.24, 0.11$) compared to a median of 0.00 mm^3 (IQR $-0.18, 0.18$) for the intervention group and this difference was statistically significant ($p=0.045$).

At surface level, the highest volume loss was observed on the buccal surfaces of the incisor teeth. The control group had a median volume change of -0.11 mm^3 (IQR $-0.29, 0.02$) whereas the intervention group had a median volume change of -0.04 mm^3 (IQR $-0.18, 0.15$) per surface and this difference was statistically significant ($p=0.041$). An overall volume loss was also observed on the palatal surfaces of the central incisors. The control group demonstrated a median volume change of -0.08 mm^3 (IQR $-0.22, -0.06$) and the intervention group a median change of -0.06 mm^3 (IQR $-0.26, 0.18$) and this difference was not statistically significant ($p=0.69$).

Interestingly, the occlusal surfaces of the lower first molars reported an overall gain in volume. The control group had a median volume change of 0.06 mm^3 (IQR $0, 1.1$) and the intervention group 0.09 mm^3 (IQR $0.1, 1.1$)) but this difference was not statistically significant ($p=0.542$).

Figure 49: Volume change analysis (mm³)

	Control Group		Intervention Group		
	Median	IQR	Median	IQR	p value
Patient Level Analysis	-0.09	(-0.14, 0.05)	-0.02	(-0.08, 0.09)	0.078
Combined Surface Level Analysis	-0.06	(-0.24, 0.11)	0.00	(-0.18, 0.18)	0.045*
Buccal Surfaces of Central Incisors	-0.11	(-0.29, 0.02)	-0.04	(-0.18, 0.15)	0.041*
Palatal Surfaces of Central Incisors	-0.08	(-0.22, -0.06)	-0.06	(-0.26, 0.18)	0.690
Occlusal Surfaces of Lower First Molars	0.06	(0.00, 1.10)	0.09	(0.10, 1.10)	0.542

5.4.6.2 Profilometric analysis

5.4.6.2.1 Profilometric change

At patient level, an overall profilometric gain was observed for the control group (median 1.3 μm (IQR 0.3, 2.6)) and the intervention group (0.3 μm (IQR -0.8, 2.0)) and this difference was not statistically significant ($p=0.076$).

At surface level, the median profilometric change was 0.5 μm for both the control group (IQR 0, 1.1) and the intervention group (IQR 0.1, 1.1) with no statistical differences observed ($p=0.245$).

When analysed further by surface, there were no clear patterns detected. On the buccal surfaces of the central incisors, the control group had a profilometric change of 1.1 μm (IQR -0.3, 5.4) compared to 0.8 μm (-2.7, 4.2) for the intervention group. On the palatal surfaces of the central incisors the control group demonstrated a profilometric change of 0.5 μm (IQR -1.5, 3.6) compared to 2.3 μm (0, 5.1) for the intervention group. No significant differences between groups were noted for the buccal or palatal incisors. The occlusal surfaces had a negative profilometric change (overall loss) with a median of -0.3 μm (IQR -2.5, 2.4) for the controls and for the intervention group -1.9 μm (IQR -3.2, 0.3) and this difference was statistically significant ($p=0.046$).

Figure 50: Profilometric change (μm)

	Control Group		Intervention Group		
	Median	IQR	Median	IQR	p value
Patient Level Analysis	1.3	(0.3, 2.6)	0.3	(-0.8, 2.0)	0.076
Combined Surface Level Analysis	0.5	(0, 1.1)	0.5	(0.1, 1.1)	0.245
Buccal Surfaces of Central Incisors	1.1	(-0.3, 5.4)	0.8	(-2.7, 4.2)	0.096
Palatal Surfaces of Central Incisors	0.5	(-1.5, 3.6)	2.3	(0, 5.1)	0.061
Occlusal Surfaces of Lower First Molars	-0.3	(-2.5, 2.4)	-1.9	(-3.2, 0.3)	0.046*

5.4.6.2.2 *Profilometric Loss*

At patient level, the profilometric loss was 18 μm (IQR 13, 22) for the control group and 17 μm (14, 21) for the intervention group, which was not statistically different ($p=0.560$).

At surface level, the mean profilometric loss for the control group was 14 μm (IQR 10, 23) and 15 μm (10, 21) for the intervention group and was not statistically different ($p=0.975$).

The buccal surfaces (Figure 51) of the controls had a median profilometric loss of 11 μm (IQR 8, 17) and intervention group 12 μm (IQR 9, 18), and this was not statistically significant different ($p=0.286$). The occlusal surfaces of the lower first molars were observed to have the greatest profilometric loss for the control group with a median of 18 μm (IQR 14, 26) and the intervention group: a median of 20 μm (IQR 12, 23), but no statistical differences were observed ($p=0.482$).

Figure 51: Profilometric loss (μm)

	Control Group		Intervention Group		
	Median	IQR	Median	IQR	p value
Patient Level Analysis	18	(13,22)	17	(14,21)	0.560
Combined Surface Level Analysis	14	(10,23)	15	(10,21)	0.975
Buccal Surfaces of Central Incisors	11	(8,17)	12	(9,18)	0.286
Palatal Surfaces of Central Incisors	15	(10,22)	16	(10,20)	0.960
Occlusal Surfaces of Lower First Molars	18	(14,26)	20	(12,23)	0.482

5.4.6.2.3 *Maximum Point Loss*

At patient level (Figure 52) the control group had a median maximum point loss recorded of 137 μm (IQR 108, 171) and the intervention group 149 μm (IQR 98, 172), with no statistically significant differences ($p=0.911$).

At the surface level, the median maximum point loss detected for the control group was 106 μm (IQR 72, 182) and 110 μm (IQR 64, 162) for the intervention group with no statistically significant differences ($p=0.668$).

The lowest median maximum point loss was observed for the buccal surfaces of the central incisors in the control group: with a median of 76 μm (IQR 50, 99) and for the intervention group was 60 μm (IQR 41, 94), $p=0.244$. The highest loss was observed on the occlusal surfaces of the lower first molars of control group: 169 μm (IQR 109, 269) and the intervention group: 168 μm (IQR 129, 339), ($p=0.616$). For the palatal surfaces the control group was 110 μm (IQR 86, 168), and intervention group: 117 μm (IQR 90, 151), $p=0.164$.

Figure 52: Maximum point loss (μm)

	Control Group		Intervention Group		
	Median	IQR	Median	IQR	p value
Patient Level Analysis	137	(108,171)	149	(98,172)	0.911
Combined Surface Level Analysis	106	(72,182)	110	(64,162)	0.668
Buccal Surfaces of Central Incisors	76	(50,99)	60	(41, 94)	0.244
Palatal Surfaces of Central Incisors	110	(86,168)	117	(90,151)	0.164
Occlusal Surfaces of Lower First Molars	169	(109,269)	168	(129,339)	0.616

5.5 DISCUSSION

This is the first clinical study to show a statistically significant difference in erosive wear progression after a dietary intervention. Other studies have reported significant erosive tooth wear progression associated with gastro-oesophageal reflux symptoms (Rodriguez *et al.* 2012a; Tantbirojn *et al.* 2012), vomiting (Rodriguez *et al.* 2012a) and attritional wear (Molnar *et al.* 1983; Lambrechts *et al.* 1989; Pintado *et al.* 1997).

Furthermore, this is the first study to demonstrate that a dietary intervention changed intake of acidic foods and drinks. Although both groups reported decreased consumption of dietary acids ($p < 0.001$), only those in the intervention group reduced the frequency of dietary acid intake between meals ($p = 0.048$) and experienced a median volume loss of 0 mm^3 (IQR $-0.18, 0.18$) compared to a median of -0.06 mm^3 IQR $(-0.24, 0.11)$ in the control group ($p = 0.045$). This implies that the behaviour change intervention was successful and this impacted on the progression of erosive wear. This follows the findings from the previous chapter which reported a higher risk associated when dietary acids were consumed between meals.

The use of behaviour change interventions to prevent erosive tooth wear progression is novel. Gollwitzer distinguished between goal and implementation intentions (Gollwitzer and Sheeran 2006). Goal intentions are linked to motivation to make the behaviour change whereas implementation intention aim to develop a plan for performing the behaviour change e.g. in this situation instead of X I will do Y. This type of planning and self-monitoring behaviour has been shown to increase adherence to flossing (Schüz *et al.* 2006; Schüz *et al.* 2009; Suresh *et al.* 2012).

Results observed from this study support the evidence that if-then planning has a positive outcome (Schüz *et al.* 2009).

A possible confounding factor is that the customisation of advice has also been reported to offer improved outcomes over generalised advice in other fields of diet change (Bradbury *et al.* 2006; Zare Javid *et al.* 2014). Two randomised controlled trials aiming to increase fresh fruit and vegetable consumption, investigated a customised diet advice compared to standard of care diet advice. Both studies observed no difference in the control groups at the end of the trials whereas the intervention groups had statistically increased fruit and vegetable consumption (Bradbury *et al.* 2006; Zare Javid *et al.* 2014). In this trial both groups were subjected to a detailed dietary erosive wear risk factor questionnaire and so both were aware that an erosive diet was the source of their wear which was being monitored. One could argue that the information provided to the control group was also customised. Despite this, the intervention group managed to statistically reduce their tooth wear to a greater extent than the control group.

There were no statistical differences observed between groups for change in the timing of tooth brushing. This may reflect the relatively small sample size.

Although a greater number of participants in the intervention group reported to have stopped brushing within 10 minutes of consuming a dietary acid the difference was statistically significant. It may also reflect that behaviours which are habitual (performed frequently in consistent contexts) are more difficult to change (Webb and Sheeran 2006). An increase in sample size is needed to investigate these preliminary findings.

There were no statistical differences in BEWE scores between visits. This supports other studies that report no difference in the clinical monitoring of tooth wear using indices over short time periods but have reported differences using laboratory equipment (Chadwick *et al.* 2005; Al-Omiri *et al.* 2013). This confirms the necessity for more precise methods of detecting small changes in erosive wear over relatively short time periods.

The mean volume of tooth tissue lost over a 6-month period for this cohort of patients was 0.035mm³ (IQR -0.20, 0.14) per surface investigated. The only other research group investigating volumetric measurements, over 12-month period, observed comparable volume loss of 0.04mm³ (SD = 0.25) (Pintado *et al.* 1997). Pintado *et al.* used contact stylus profilometry with a diameter of 300 µm and data point collected every 50 µm and developed their own superimposition software to align using root mean square differences. The slightly reduced wear observed in their study may be a reflection of the younger age group (aged 18-22, n=18) with no apparent risk factors.

Interesting observations were made when assessing the different tooth wear measurement methods. Volume change measurement was able to detect differences between the two groups over a 6-month observation period. In contrast there was no difference in other profilometric measurements between groups. This has also been observed in other studies whereby few differences were observed when measuring the average profilometric loss over the entire surface of the tooth (Chadwick *et al.* 2005; Rodriguez *et al.* 2012a). There are three possible reasons for this. The surface matching software aligns the surfaces by minimising root mean square differences between scanned data points. The attempt to then

measure these differences which the software has attempted to minimise is flawed. Furthermore, if there is a large deviation in a localised area, the whole surface alignment may be tilted to accommodate this gain (Mitchell *et al.* 2004). The subsequent interpolated data results in overly conservative estimations with large standard deviations (Mitchell *et al.* 2004). Additionally, if wear occurs in a localised area, as was observed in several cases within this study (Figure 37), the wear is averaged over every data point and the effect becomes minimised.

Volume change measurement uses the superimposition process solely as a method to create a digital reference from which future measurements can be compared. This avoids any interpolation errors. It is unclear in previous studies using volume change whether they have used interpolated data or original data (Pintado *et al.* 1997; Tantbirojn *et al.* 2012). This method of analysis may be more accurate for use in future studies.

Interesting observations were made when assessing reproducibility on the different surfaces. The overall profilometric loss error in this study (11 μm , (IQR 9, 18)) is similar to values reported by Rodriguez *et al.* 2012 ($\pm 15 \mu\text{m}$). However, this is the first study to the author's knowledge to analyse reproducibility according to different surfaces. Measurement error for profilometric loss was observed to be 9 μm for the incisors increasing significantly to 22.5 μm for the molars ($p < 0.001$). Similar differences in errors were observed for all other measurement parameters. This large measurement error in reproducibility cannot be attributed to the materials. The materials used within this study have been extensively validated and measurement errors reported (Rodriguez and Bartlett 2011).

Polyvinylsiloxane impressions and type 4 dental stone mixed in a vacuum have

repeatedly been observed to result in the least measurement error and increased reproducibility (Price *et al.* 1991; Chadwick *et al.* 2002; Rodriguez and Bartlett 2011). The laser profilometer used had a spot size of 30 μm and a sensor resolution of 0.1 μm . Previous work by our group has observed this laser to be repeatable to 1.6 μm over a 25 mm range (Rodriguez *et al.* 2009). However, this laser is unable to measure undercuts. As the occlusal surfaces of molars are curved with additional topographical features, this may pose difficulties when attempting to capture detail at a micron level. Detail may be lost when taking the impression, casting it in stone (ensuring no slight air blows or stone excess which may be invisible to the naked eye), and in the superimposition process whereby increased features are more difficult to align precisely. It could also be a result of different tilts when scanning the casts (Lambrechts *et al.* 1984) or varying amounts of cast porosity meaning deeper penetration of the laser (Whitehead *et al.* 1999). This error could also be due to clinical parameters. The thickness of the salivary pellicle can range from 0.1-1.3 μm (Hannig and Balz 1999) and the thickness of the salivary film has been reported to be between 60-90 μm (Watanabe and Dawes 1990). Although attempts were made to completely isolate and dry the index teeth, the improved reproducibility for the anterior teeth may have been due to improved isolation and control over impression positioning in the anterior area, in addition to the more favourable topography of smoother surfaces. Future studies could consider the use of smooth surfaces to save resources and reduce error. The majority of the wear was observed on the buccal surfaces of the central incisors. The intervention group had significantly less volume loss on this surface than the control group ($p=0.041$). Increased wear on the buccal surfaces of the dentition have been reported in other studies, using epidemiological indices as opposed to

quantitative tooth wear analysis (Bartlett, Fares, *et al.* 2011); although it is generally reported to be on the canines and premolars (Lussi and Schaffner 2000). A study used the Eccles and Jenkins index 1979 to investigate erosive wear in 277 institutionalised alcoholics at risk of both intrinsic and extrinsic erosion (Teixeira *et al.* 2016). They observed that although the occlusal surfaces were more commonly affected and associated with gastric symptoms, dentine exposure was greatest in the buccal surfaces of the incisors and associated with a high daily alcohol intake. Unfortunately there are no quantitative data available. Conversely, Moazzez *et al.* observed in a clinical investigation reported that the control group was more likely to maintain a lower pH for longer on the buccal surfaces of the central incisors than the erosive wear group (Moazzez *et al.* 2000). It is worth noting however that the control group spent longer drinking their drink (median 8 minutes) compared to the erosive wear group (median 5 minutes).

The buccal surfaces of the central incisors were also observed to have the lowest profilometric loss and maximum point loss values compared to the other surfaces investigated. The acid may affect a larger surface area on the buccal surface rather than smaller localised areas and further studies are needed to investigate this. In contrast, the occlusal surfaces of the lower first molars exhibited an overall gain in volume but the highest amount of profilometric loss and maximum point loss. This finding is more difficult to explain and may be attributed to the reproducibility error on molars discussed previously or that localised lesions may be more likely to occur on molars due to the complex topography of the surface.

There are biases within this study design that need to be considered. Self-reported outcome measures are subject to reporting bias. The interviewer-led questionnaire

provides extensive detail about risk factors associated with dietary acid intake. There is a possibility that both groups were able to deduce risk factors associated with erosive tooth wear and chose to act or report differently. Participants were in a clinical setting and aware of their condition. They may have under-reported dietary acid intake due to social desirability bias. Additionally, the Hawthorne effect is a social phenomenon where participants act differently when they know they are being observed (Sedgwick and Greenwood 2015). Although participants were blinded to which intervention they received, both groups were aware that their tooth wear was being monitored and may have changed behaviour as a result of this and not the intervention. Having stated these limitations, it is promising that the clinical outcomes reflect the self-reported behavioural outcomes. Ideally, long term follow-up would occur to assess adherence to the behaviour change.

An attempt was made to address confounding factors. The randomisation process successfully balanced the groups with no statistical differences between ages, gender, baseline level of erosive wear as measured by the BEWE or numbers of surfaces analysed. Both the participants and the investigator performing analysis were blinded to the allocation group. The known clinical confounding factors of xerostomia, parafunction or intrinsic acid damage were excluded. Statistical analysis was performed by a statistician. However, there are additional limiting factors. The sample size, although sufficiently powered, was relatively small. This is due to the intensive workload required to source, take impressions and analyse data from each patient. There was a relatively low dropout rate of 5%; however per protocol statistical analysis was used in this trial. Intention to treat analysis of the data was not used as a follow up appointment was needed to generate both the

dietary change data and the tooth wear progression data. Patients were also recruited from a hospital population. This group may be more aware of their condition and subsequently more motivated to change their behaviour. Although differences were observed in this cohort of patients, further trials in the general population are needed to assess the generalisability of the results.

5.6 CONCLUSION

Implementation planning, as a dietary change intervention, successfully improved the dietary clinical and self-reported behavioural outcomes measured in this trial. Reducing dietary acid intake between meals was associated with a reduction in the volumetric loss of dental tissue over a 6-month observation period in this cohort of patients, therefore the first and third null hypotheses were rejected. In contrast, tooth brushing behaviours were not statistically significantly altered by the intervention and there is insufficient evidence to reject the second null hypothesis. Volume change measurement on the buccal surfaces of the central incisors appears to be promising as a method for future intervention assessment. Further work is required in general practice to assess if the results of this trial are generalisable to the general population.

CHAPTER 6: GENERAL DISCUSSION AND IMPLICATIONS

This thesis identified high-risk behaviours associated with dietary acid consumption. Consistently stronger associations with erosive tooth wear were observed when dietary acids were consumed between meals on a daily basis. This naturally led in to an interventional study that reported the frequency of dietary acid intake between meals reduced tooth wear progression. The behaviour change intervention was applied at a single sitting lasting less than 5 minutes with no further advice until reassessment 6 months later. These findings should assist general practitioners to identify risk specific factors and apply them to change behaviour and reduce the progression of erosive tooth wear.

There are several other novel findings within this thesis. Consuming fruit over periods greater than 10 minutes, twice daily consumption of acidic drinks between meals and the effect of drinking habits prior to swallowing were related to severe erosive tooth wear. This highlights the importance of assessing complete patterns of dietary acid intake rather than focusing on what is being consumed and how often. Brushing teeth within 10 minutes of consuming a dietary acid was not associated with erosive wear. The laboratory study highlighted the confounding influence of when to use a standardised mouthrinse and it suggests the formulation of fluoride might influence progression.

The methodology employed to measure erosive wear in vivo using profilometry and surface matching software was developed for this thesis. However, the technique is challenging and there were variations in data. The change in volume, particularly on the buccal surfaces of central incisors was the most reliable measurement to assess progression. Ideally, the occlusal surface of lower first

molars, which are the most commonly site, would be useful to monitor wear..

However, until the technology and methodology improve, the buccal surfaces of central incisors is the most appropriate surface to use to measure clinical progression of erosive tooth wear.

Overall, findings observed in this thesis imply that prevention should be focused on minimising dietary acids between meals and considering the use of stannous fluoride.

CHAPTER 7: SUGGESTIONS FOR FUTURE WORK

There are several findings that warrant further investigation.

The finding that dental professionals can influence behaviour change is promising and generates an entire field of research. The protocol within this thesis was performed by a single operator, in a hospital environment with a single cohort of patients. Future work should aim to repeat the study using multiple operators and different patient populations. It would be interesting to investigate if behaviour change interventions were more successful at reducing erosive wear than clinical interventions such as high concentration fluoride placement or sealant placement.

The majority of dental diseases are preventable (Birch *et al.* 2015) and commonly caused by unfavourable behaviours. Future research could also investigate the effectiveness of behaviour change interventions in other dental diseases. Adopting a multi-disciplinary approach, the role of the dentist in promoting behaviour change for non-oral diseases could also be investigated. Ideally longer monitoring periods would be observed and inter-disciplinary clinical outcomes would be used to assess adherence to the behaviour change.

Further research is also needed to refine the use of profilometry and surface matching software when monitoring tooth wear in vivo. Although the results of the present study are promising and were able to detect small changes, the standard deviations and inherent error measurements were large. Future research needs to collaborate with computer programmers to design software that would refine the process and improve the accuracy of measurement. The ultimate aim of the process is to allow for more accurate assessment of interventions over a shorter time period.

Although it was observed that stannous fluoride was optimally applied before the erosive challenge with a simple experimental design, this needs to be tested with a more clinically relevant design. The relationship between fluoride, abrasion and erosive tooth wear has not been fully answered. The in vitro experiment in Chapter 2 would ideally be repeated including toothpastes, abrasion and a salivary pellicle in the experimental design. The inclusion of the pellicle is important as studies have associated the thickness of the salivary pellicle with protection against erosive wear (Amaechi *et al.* 1999). Toothbrush abrasion does not remove the pellicle but reduces its size (Hannig and Balz 1999), and it would be interesting to see if the protection offered by the fluoride compensates for the reduced pellicle thickness. This would further elucidate whether it is optimal to brush before or after an erosive challenge with either sodium or stannous fluoride.

APPENDICES

8.1 INCLUSION/EXCLUSION CRITERIA QUESTIONNAIRE STUDY

Inclusion criteria

1. Severe tooth wear with a BEWE score of 12 and at least one score of 3 in a quadrant
2. Adult 18 years or older
3. No missing anterior teeth
4. Minimum of at 10 teeth in the upper and 10 teeth in the lower jaw
5. No anterior crowns/ bridges or implants
6. Written consent to the study

Exclusion criteria

1. Pregnancy
2. Participation in other research within 30 days
3. Unable to speak or understand English
4. Presence of periodontal disease or caries on more than one tooth

The inclusion of patients is aimed at those who present with tooth wear and no other dental conditions such as missing teeth or periodontal disease or caries. The control participants will have the same inclusion and exclusion criteria apart from an absence of tooth wear.

8.2 DIETARY ACID QUESTIONNAIRE (PRINTABLE FORMAT)

BEWE

Upper right
Upper centre
Upper left
Lower right
Lower centre
Lower left

Age

Gender (Female =1, Male =0)

Sensitivity

Do you consider yourself as currently suffering from sensitive teeth?(Yes = 1)

Do you regularly suffer from sensitive teeth when? 1=While brushing teeth

2= With cold weather/air 3= To touch 4=With hot things 5=To sweet

6= With cold drinks/ice 7=Other

Brushing

Cold weather/air

Touch

Hot things

Sweet things

Cold drinks/ice

Other

On a scale from 0-10 how would you rate the pain from your sensitive teeth?

What toothpaste do you use?

Do you currently use de-sensitising toothpaste? (Yes = 1)

Brushing

Do you use an electric toothbrush? (Yes =1)

If not do you use a soft (1), medium (2) or hard (3) toothbrush?

Do you brush your teeth for ≥ 2 min (1) or < 2 min (2)

Do you brush your teeth within 10 minutes after eating or drinking something acidic
(Yes = 1)

How many times a day do you brush your teeth?

When do you brush your teeth in relation to:

1= 1 hour before **2**= 30min-1hour before **3**= < 10 min before **4**= 1 hour after

5=30min- 1hour after **6**=<10 min after

Breakfast

Lunch

Dinner

For Breakfast do you have any of the following:

Fruit Juice

Fruits

Citrus Fruits

FRUITS

How many times do you eat fruit per day? 1, 2, 3... per day, 555=<1 per day but >1/week

How long would it take you eat that fruit? <5 min 5-10 min >10 min

Citrus Fruits

How often would you consume it with a meal? 1, 2, 3... per day, 555=<1 per day but >1/week

How often would you consume it outside a meal? 1, 2, 3... per day, 555=<1 per day but >1/week

How long would it take you eat that fruit? <5 min 5-10 min >10 min

Apples

How often would you consume it with a meal? 1, 2, 3... per day, 555=<1 per day but >1/week

How often would you consume it outside a meal? 1, 2, 3... per day, 555=<1 per day but >1/week

How long would it take you eat that fruit? <5 min 5-10 min >10 min

Grapes

How often would you consume it with a meal? 1, 2, 3... per day, 555=<1 per day but >1/week

How often would you consume it outside a meal? 1, 2, 3... per day, 555=<1 per day but >1/week

How long would it take you eat that fruit? <5 min 5-10 min >10 min

Berries

How often would you consume it with a meal? 1, 2, 3... per day, 555=<1 per day but >1/week

How often would you consume it outside a meal? 1, 2, 3... per day, 555=<1 per day but >1/week

How long would it take you eat that fruit? <5 min 5-10 min >10 min

Other Fruits

How often would you consume it with a meal? 1, 2, 3... per day, 555=<1 per day but >1/week

How often would you consume it outside a meal? 1, 2, 3... per day, 555=<1 per day but >1/week

How long would it take you eat that fruit? <5 min 5-10 min >10 min

DRINKS

How many times a day do you have an acidic drink? 1, 2, 3... per day,
555 =<1 per day but >1/week

How long would it take you to drink it? <5 min 5-10 min >10 min

Fruit Juice

How often would you drink it with a meal? 1, 2, 3... per day, 555=<1 per day
but >1/week

How often would you drink it outside a meal? 1, 2, 3... per day, 555=<1 per day
but >1/week

How long would it take you to drink it? <5 min 5-10 min >10 min

Do you most commonly use a cup, glass, bottle or can?

Do you tend to sip, swish or hold your drinks in your mouth?

How many glasses would you have at one sitting? 1, 2, 3... per day, 555=<1 per
day but >1/week

Fizzy/Soft Drinks

How often would you drink it with a meal? 1, 2, 3... per day, 555=<1 per day
but >1/week

How often would you drink it outside a meal? 1, 2, 3... per day, 555=<1 per day but
>1/week

How long would it take you to drink it? <5 min 5-10 min >10 min

Do you most commonly use a cup, glass, bottle or can?

Do you tend to sip, swish or hold your drinks in your mouth?

How many glasses would you have at one sitting? 1, 2, 3... per day, 555=<1 per day
but >1/week

Other Acidic Drinks

How often would you drink it with a meal? 1, 2, 3... per day, 555=<1 per day but >1/week

How often would you drink it outside a meal? 1, 2, 3... per day, 555=<1 per day but
>1/week

How long would it take you to drink it? <5 min 5-10 min >10 min

Do you most commonly use a cup, glass, bottle or can?

Do you tend to sip, swish or hold your drinks in your mouth?

How many glasses would you have at one sitting? 1, 2, 3... per day, 555=<1 per day
but >1/week

Other

Do you regularly use a straw?

Do you frequently suffer from any of the following conditions?

Heartburn

Vomiting

Chest pain

Regurgitation

Clenching teeth

Grinding teeth

Do you regularly take medication for heartburn?

Do you ever suffer from dry mouth?

8.3 QUESTIONNAIRE RESULTS: FREQUENCIES AND CRUDE ANALYSIS

Presented here are the frequencies and crude analysis (logistic regressions controlling for age and gender) for questions not reported in the main text. These include the stimuli to which most frequently resulted in self-reported dental hypersensitivity, the use of de-sensitising toothpaste, hypersensitivity pain intensity and patterns of consumption for the different types of fruit and acidic drinks investigated.

8.3.1 DENTAL HYPERSENSITIVITY

Figure 53: Self-reported dental hypersensitivity

	Erosive Wear Patients		Controls	
	n	%	n	%
Of those with self-reported hypersensitivity, how intense is their pain on a scale of 0-10 (IQR)?	4 (3,6)		4 (3,6)	
Self -reported sensitivity when:				
While brushing teeth	15	(5%)	31	(10.3%)
Cold weather/air	94	(31.3%)	49	(16.3%)
Touch	28	(9.3%)	5	(1.7%)
Hot things	63	(21%)	33	(11%)
Sweet things	61	(20.3%)	25	(8.3%)
Cold drinks/ice	138	(46%)	96	(32%)
Other	23	(7.7%)	5	(1.7%)
Do they use de-sensitising toothpaste?				
Yes	146	(48.7%)	105	(35%)
No	154	(51.3%)	195	(65%)

	OR	95% CI	p value
Does the patient regularly suffer from sensitive teeth with:			
Brushing teeth	2.4	(1.25 - 4.58)	0.008
Cold weather/air	2.7	(1.78 - 4.02)	<0.001
Touch	6.6	(2.49 - 17.43)	<0.001
Hot things	2.4	(1.52 - 3.88)	<0.001
Sweet things	3.1	(1.88-5.18)	<0.001
Cold drinks/ice	2.0	(1.42 - 2.85)	<0.001
Other stimuli	4.9	(1.85 - 13.34)	<0.001
Do they use de-sensitising toothpaste?			
No	1		
Yes	1.9	(1.34-2.61)	<0.001

8.3.2 FRUIT CONSUMPTION PATTERNS

Figure 54: Further analysis of different types of fruit

		Erosion Patient		Control	
		n	%	n	%
Fruit intake patterns					
Citrus	With a meal	43	(14.3%)	36	(12%)
	Outside a meal	111	(37%)	42	(14%)
Apples	With a meal	24	(8%)	33	(11%)
	Outside a meal	138	(46%)	94	(31.3%)
Grapes	With a meal	6	(2%)	6	(2%)
	Outside a meal	44	(14.7%)	25	(8.3%)
Berries	With a meal	27	(9%)	30	(10%)
	Outside a meal	13	(4.3%)	19	(6.3%)
Other fruits	With a meal	39	(13%)	59	(19.7%)
	Outside a meal	98	(32.7%)	94	(31.3%)
Time taken to eat fruits					
Citrus	<5 min	79	(26.3%)	72	(24%)
	≥5 min	62	(20.7%)	5	(1.7%)
Apples	<5 min	120	(40%)	113	(37.7%)
	≥5 min	38	(12.7%)	15	(5%)
Grapes	<5 min	28	(9.3%)	28	(9.3%)
	≥5 min	21	(7%)	5	(1.7%)
Berries	<5 min	25	(8.3%)	34	(11.3%)
	≥5 min	15	(5%)	14	(4.7%)
Other fruits	<5 min	98	(32.7%)	133	(44.3%)
	≥5 min	36	(12%)	12	(4%)

		OR	95% CI	p value
Fruit Intake Patterns				
Citrus				
	With a meal	1.21	(0.75 – 1.96)	0.434
	Outside a meal	3.87	(2.57 – 5.83)	<0.001
Apples				
	With a meal	0.67	(0.38 – 1.17)	0.158
	Outside a meal	1.96	(1.40 – 2.76)	<0.001
Grapes				
	With a meal	1.03	(0.33 – 3.29)	0.954
	Outside a meal	2.04	(1.21 – 3.46)	0.008
Berries				
	With a meal	0.96	(0.55 – 1.68)	0.888
	Outside a meal	0.78	(0.37 – 1.63)	0.509
Other fruits				
	With a meal	0.59	(0.37 – 0.92)	0.021
	Outside a meal	1.04	(0.73 – 1.47)	0.827
Time taken to eat fruits				
Citrus				
	<5 min	1		
	≥5 min	12.94	(4.87 – 34.40)	<0.001
Apples				
	<5 min	1		
	≥5 min	2.66	(1.37 – 5.17)	0.004
Grapes				
	<5 min	1		
	≥5 min	4.73	(1.53 – 14.64)	0.007
Berries				
	<5 min	1		
	≥5 min	1.41	(0.57 – 3.48)	0.456
Other fruits				
	<5 min	1		
	≥5 min	4.61	(2.25 – 9.43)	<0.001

8.3.3 ACIDIC DRINK CONSUMPTION PATTERNS

Figure 55: Further analysis of different types of acidic drinks

		Erosion Patient		Control	
		n	%	n	%
Juice with a meal					
	0	192	(64%)	224	(74.7%)
	<1/day but >1/week	5	(1.7%)	19	(6.3%)
	1/day	73	(64%)	46	(15.3%)
	2+/day	30	(24.3%)	11	(3.7%)
Juice outside a meal					
	0	174	(58%)	229	(76.3%)
	<1/day but >1/week	15	(5%)	13	(4.3%)
	1/day	53	(17.7%)	42	(14%)
	2+/day	58	(19.3%)	16	(5.3%)
Fizzy drinks with a meal					
	0	229	(76.3%)	264	(88%)
	<1/day but >1/week	13	(4.3%)	11	(3.7%)
	1/day	28	(9.3%)	24	(8%)
	2+/day	30	(10%)	1	(0.3%)
Fizzy drinks outside a meal					
	0	197	(65.7%)	259	(86.3%)
	<1/day but >1/week	21	(7%)	12	(4%)
	1/day	41	(13.7%)	19	(6.3%)
	2+/day	41	(13.7%)	10	(3.3%)
Other acidic drinks with a meal					
	0	237	(53.7%)	229	(76.3%)
	<1/day but >1/week	29	(23%)	53	(17.7%)
	1/day	25	(13.3%)	16	(5.3%)
	2+/day	9	(10%)	2	(0.7%)
Other acidic drinks outside a meal					
	0	161	(53.7%)	188	(62.7%)
	<1/day but >1/week	69	(23%)	69	(23%)
	1/day	40	(13.3%)	39	(13%)
	2+/day	30	(10%)	4	(1.3%)

Variable	OR	95% CI	p value
Juice with a meal			
0 1			
<1/day but >1/week	0.31	(0.11 – 0.84)	0.022
1/day	1.85	(1.21 – 2.83)	0.004
2+/day	3.30	(1.59 – 6.82)	0.001
Juice outside a meal			
0 1			
<1/day but >1/week	1.61	(0.74 – 3.52)	0.232
1/day	1.77	(1.12 – 2.80)	0.015
2+/day	4.92	(2.71 – 8.92)	<0.001
Fizzy drinks with a meal			
0 1			
<1/day but >1/week	1.40	(0.61 – 3.22)	0.428
1/day	1.33	(0.74 – 2.38)	0.339
2+/day	33.82	(4.55 – 251.17)	0.001
Fizzy drinks outside a meal			
0 1			
<1/day but >1/week	2.29	(1.08-4.86)	0.03
1/day	2.88	(1.61- 5.14)	<0.001
2+/day	5.13	(2.48 – 10.61)	<0.001
Other acidic drinks with a meal			
0 1			
<1/day but >1/week	0.51	(0.31 – 0.84)	0.008
1/day	1.53	(0.79 – 2.95)	0.210
2+/day	4.35	(0.92 – 20.65)	0.064
Other acidic drinks outside a meal			
1			
0 1			
<1/day but >1/week	1.11	(0.74-1.67)	0.603
1/day	1.22	(0.75-2.000)	0.424
2+/day	9.15	(2.14-26.73)	<0.001

8.3.4 TIME TAKEN TO DRINK ACIDIC DRINKS

Figure 56: Time taken to consume acidic drinks

		Erosion Patient		Control	
		n	%	n	%
Juices	<10 min	106	(35.3%)	88	(29.3%)
	≥10 min	71	(23.7%)	34	(11.3%)
Fizzy drinks	<10 min	49	(16.3%)	34	(11.3%)
	≥10 min	78	(26%)	35	(11.7%)
Other acidic drinks	<10 min	27	(9%)	20	(6.7%)
	≥10 min	130	(43.3%)	120	(40%)

		OR	95% CI	p value
Juices	<10 min	1		
	≥10 min	1.87	(1.13 – 3.11)	0.015
Fizzy drinks	<10 min	1		
	≥10 min	1.59	(0.87-2.88)	0.130
Other acidic drinks	<10 min	1		
	≥10 min	0.75	(0.39 -1.43)	0.381

8.3.5 CONTAINERS FOR INDIVIDUAL ACIDIC DRINKS

Figure 57: Containers for acidic drinks

		Erosion Patient		Control	
		n	%	n	%
Juices					
	Cup	10	(3%)	13	(3.9%)
	Glass	144	(48%)	105	(35%)
	Bottle	23	(8%)	5	(1.5%)
	Can	0	(0%)	0	(0%)
Fizzy drinks					
	Cup	5	(2%)	0	(0%)
	Glass	46	(15%)	20	(7%)
	Bottle	29	(10%)	11	(4%)
	Can	51	(17%)	36	(12%)
Other acidic drinks					
	Cup	28	(9%)	14	(5%)
	Glass	110	(37%)	119	(40%)
	Bottle	12	(4%)	7	(2.1%)
	Can	1	(0.3%)	1	(0.3%)

		OR	95% CI	p value
Juices				
	Cup/Glass	1		
	Bottle	3.54	(1.29 – 9.70)	0.014
	Can	-	-	-
Fizzy drinks				
	Cup/Glass	1		
	Bottle	1.04	(0.44 – 2.50)	0.924
	Can	0.56	(0.287 – 1.11)	0.096
Other acidic drinks				
	Cup/Glass	1		
	Bottle	0.97		0.459
	Can	0.56	(0.55 – 3.84)	0.973
			(0.06 – 15.88)	

8.3.6 HABITS FOR ACIDIC DRINKS

Figure 58: Habits for acidic drinks

		Erosion Patient		Control	
		n	%	n	%
Juices					
	Sip	23	(8%)	3	(0.3%)
	Swish	16	(5%)	0	(0%)
	Hold	13	(4%)	2	(0.7%)
Fizzy drinks					
	Sip	18	(5.4%)	3	(0.9%)
	Swish	11	(4.3%)	1	(0.3%)
	Hold	7	(2.1%)	0	(0%)
Other acidic drinks					
	Sip	30	(10%)	5	(1.5%)
	Swish	9	(3%)	1	(0.3%)
	Hold	7	(2.1%)	2	(0.6%)

		OR	95% CI	p value
Juices				
Drinks juice but no alternative drinking habit		1		
	Sip	1.66	(1.18-2.35)	0.004
	Swish	12.61	(3.66-43.45)	<0.001
	Hold	-	-	-
Fizzy drinks				
Drinks fizzy drinks but no alternative drinking habit		1		
	Sip	1.93	(1.32-2.83)	0.001
	Swish	8.14	(2.33-28.46)	0.001
	Hold	12.47	(1.84-114)	0.011
Other acidic drinks				
Drinks other acidic drinks but no alternative drinking habit		1		
	Sip	0.86	(0.61-1.22)	0.402
	Swish	7.58	(2.84-20.26)	<0.001
	Hold	9.66	(1.19-78.41)	0.034

8.4 INCLUSION/EXCLUSION CRITERIA FOR RANDOMISED CONTROLLED TRIAL

Inclusion Criteria

1. Severe tooth wear with a BEWE score of 3 on the occlusal surface of the first lower molars or incisal/buccal surface of the upper central incisor.
2. This wear will be as a result of a high acid diet i.e. as at least two dietary acidic challenges a day.
3. Adult 25-70 years old.
4. Minimum of at least 10 occluding tooth pairs (i.e. at least 10 upper teeth which bite against 10 lower teeth) – including the opposing upper molars and lower incisors
5. No anterior crowns/ bridges or implants opposing the lower molars or upper incisors
6. Written consent to the study

Exclusion Criteria

1. Pregnancy or breast feeding
2. Medical history likely to impact on attendance or mobility
3. Presence of periodontal disease or caries on more than one tooth. BPE score of 2 or above.
4. Unable to speak or understand English
5. Saliva diagnoses (xerostomia- dry mouth)
6. Orthodontic appliances
7. Severe dental hypersensitivity
8. Restoration of the occlusal or incisal surfaces of upper anterior teeth and first molars.
9. Have factors which could contraindicate their participation, such as any condition requiring the need for antibiotic premedication prior to a dental treatment, a condition requiring the need for long-term antibiotic use, blood thinning medications that prohibit the safe conduct of a dental cleaning or previous use of the weight loss medications.
10. Participation in other research within 30 days
11. Preferring restoration of their teeth rather than dietary intervention

8.5 ETHICAL APPROVAL FOR RANDOMISED CONTROLLED TRIAL


Health Research Authority
NRES Committee East Midlands - Nottingham 2
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 0115 883 9435

02 October 2014

Professor David Bartlett
Professor of Restorative Dentistry
Kings College London Dental Institute
Floor 25. Tower Wing
London Bridge
Se1 9RT

Dear Professor Bartlett,

Study title:	Impact of dietary advice on the progression of tooth wear
REC reference:	14/EM/1171
IRAS project ID:	158728

Thank you for your e-mail of 29 September 2014. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 24 September 2014.

Documents received

The documents received were as follows:

Document	Version	Date
Participant consent form	2	14 October 2014
Participant information sheet (PIS) [Clean and tracked]	2	14 October 2014
Other [Dietary Questionnaire Printable Format]	2	14 October 2014

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	Version	Date
Other [Researcher Script]		
Other [Dietary Advice Information]		01 November 2014
Other [Letter to participant]		01 November 2014

8.6 DIET PROMPT SHEET

Dietary advice information

To stop the damage that acidic foods are doing to your teeth you need to make small adjustments in the way you eat and drink some foods. You can do this in three ways.

1. Choose alternative snacks and drinks to reduce how often you have acidic drinks and foods.
2. When you do have acidic foods, have them with other foods, at mealtimes rather than snacks.
3. Make sure that acidic foods and drinks are in contact with your teeth for the shortest time possible. (E.g. eat things at one sitting, use a straw for drinks, not sipping or holding drinks in your mouth).

✓ Safe Snacks **BETWEEN** meals ✓



Vegetables
Breadsticks, crackers (unsalted)
Tortilla chips (baked, not fried)
Cheeses*, milk based products e.g. natural yogurt
Nuts, pumpkin seeds, sunflower seeds, hummus
Plain popcorn (not sweetened or buttered)



✓ Safe Drinks **BETWEEN** meals ✓



Water
Milk*
Tea/coffee without sugar
Herbal teas without fruits/citrus flavour



✗ Drinks to avoid **BETWEEN** meals ✗



Fizzy drinks except unflavoured sparkling water.
Fizzy diet drinks, sports and energy drinks
All juices, particularly citrus fruit juices
Smoothies
Fruit squash / cordial
Vitamin C drinks
Cider, wine, lager
Fruit teas, herbal teas with fruit / citrus



✗ Foods to avoid **BETWEEN** meals ✗









Fruits, particularly citrus fruits
Tomatoes
Vinegars particularly apple cider vinegar, pickles, ketchups



Lemon juice based salad dressing

BETWEEN MEALS

Swap this	For this	Helpful tips
Fizzy flavoured drinks	Water, Sparkling water, Milk*, Tea, Coffee	<ul style="list-style-type: none"> If you do have them, have them as infrequently as possible and over a short time period. Try not to sip, swish or hold the drinks in your mouth.
Juices, cordials 	Water, Milk*, Tea, Coffee 	<ul style="list-style-type: none"> If you are going to have juices try and drink them only with meals. Try and dilute them with more and more water to gradually wean yourself off the taste.
Wine, Beer, Cider, Spirits with mixer	It is always better to drink alcohol with meals.	<ul style="list-style-type: none"> Remember to drink alcohol sensibly Most mixers with spirits are acidic so try to cut down on these outside of meals. Try to avoid putting slices of lemons/limes in your drink.
Fruits as snacks	Vegetables – carrot sticks, celery sticks, cucumber, chopped up peppers Nuts Cheese*    	<ul style="list-style-type: none"> Try and eat fruits with meals, as a dessert. If you can, snack on vegetables. If you are going for a fruit as a snack avoid citrus fruits and apples, try and go for a banana or a plum. Try and eat the fruit over a small time period, i.e. have 10 strawberries/grapes at once rather than nibbling on one every few minutes. Try to eat any acidic foods with something that contains calcium e.g. yoghurt* or milk*
Vinegars, pickles, ketchups, lemon juice based dressings	Mayonnaise*	<ul style="list-style-type: none"> Try to reduce the amount of these you put on foods If you can avoid having them on foods in between meals

*Try to choose low-fat alternatives

Protecting your teeth against acid attack should be part of an overall balanced diet and our suggestions should help you keep to an eating pattern which is healthy – not too high in fat, salt, sugar and alcohol with plenty of vegetables and fruit at mealtimes.

8.7 IF-THEN PLAN

Making a change! Reducing the risk of acid erosion – IF/THEN planning.

What do I plan to do:

(Please tick one)

<input type="checkbox"/>	I am going to reduce how often I eat things which can cause erosion to my teeth.
<input type="checkbox"/>	I am going to reduce how often I drink things which can cause erosion to my teeth.
<input type="checkbox"/>	I am going to brush my teeth BEFORE I eat.

What I will do instead:

[illegible]

What will help me to make this change:

[illegible]

8.8 RESEARCHER SCRIPTS FOR DIETARY ADVICE

Researcher scripts to ensure dietary advice intervention are standardised.

Script for control group

Our examination has revealed that you show signs of erosion of the teeth. This is most likely to be due to a combination of the foods and drinks that you have, when you have them and when you brush your teeth.

We recommend that you cut down on the frequency of having acidic foods and drink.

Script for intervention group

Step 1: Identifying target behaviour

Resources:

- Prompt sheet – foods that have high erosive potential: List

Script:

Our examination has revealed that you show signs of erosion of the teeth. This is most likely to be due to a combination of the foods and drinks that you have, when you have them and when you brush your teeth. Our aim is to decrease the amount of erosion that is happening, and this means helping you to change these three behaviours.

To summarise from the questionnaire:

You have X number of foods during the day where there is a possibility of acid attacks on your teeth

You have Y number of drinks that can cause erosion of the teeth

You tend to clean your teeth just after eating or drinking / You always clean your teeth before eating – which is the best way to protect your teeth from erosion.

These behaviours damaged your teeth and are the behaviours we want to change to stop the damage from getting worse. From the start can I say that we don't want you to stop eating these foods, but we recommend that instead of eating them one at a time throughout the day, you try to eat them all at once at mealtimes once or twice a day.

Step 2: Behaviour specific intervention

Resources:

- Prompt sheet – IF/THEN planning

2.1 Selecting the target

Script:

Which do you think would be easier to tackle first – the foods or the drinks?
(Consider giving advice from the professional's perspective, such as "From my perspective I think we could make the biggest difference by ...")

OK, you think it would be easiest to ...

2.2 Target the frequency of foods with high erosive potential.	2.3 Target the frequency of drinks with high erosive potential.	2.4 Target brushing after an acidic attack
Looking at when you have your acidic foods, which one would be the easiest to stop?	Looking at when you have your drinks, which one would be the easiest to change?	You mentioned that you tend to clean your teeth after you have eaten. This causes problems because the acid on the surface of the teeth works with the brushing to wear away the teeth. Ideally you should clean your teeth before meals and not clean your teeth at least one hour after eating or drinking any of the foods on the list.
↓	↓	↓
<p>If you stopped that snack, what would you do instead?</p> <p>(Options are Substitution or Remove: Prompt for substitution of a non-erosive snack / eat snack at previous meal so feel fuller. If suggests alternative then prompt How would you remember to eat that instead? What would help you to remember? Would it mean taking something to work?</p> <p>If suggests "Have nothing" prompt: How easy would</p>	<p>If you changed that drink, what would you drink instead?</p> <p>(Options are Substitution or Remove: Prompt for substitution of a non-erosive drink. If suggests alternative then prompt How would you remember to drink that instead? What would help you to remember? Would it mean taking something to work or school?</p> <p>If suggests "Have nothing" prompt: How easy would that be – do you think you might find yourself getting really thirsty?</p>	<p>How easy do you think it would be to change from cleaning your teeth after to before meals?</p> <p>(What would be difficult about that? Prompt – feel odd, teeth might feel 'dirty', forgetting)</p> <p>Yes, it would feel odd at first but after a while you will get used to it, like any new habit it feels odd at first but after a while it becomes normal. (Give example e.g. new jewelry, having a mobile 'phone – before we had them it didn't feel odd not having</p>

that be – do you think you might find yourself getting hungrier later on? What would you do then? - Prompt for not snacking again, suggest eat more at previous meal to sustain hunger.)	What would you do then? - Prompt for substitution with water.)	one, but now it does etc.) (If concerned about mouth feel, prompt for alternatives) You could try using mouthwash after a meal to make your mouth feel clean – this is good because it doesn't involve the brushing.
↓	↓	↓
We have found that a useful way to remember this is to make a note on this sheet. We complete it like this (Note target on prompt sheet)	We have found that a useful way to remember this is to make a note on this sheet. We complete it like this (Note target on prompt sheet)	Now there is one exception to this – when you brush your teeth last thing at night. Once you have cleaned your teeth at night you should not have anything else to eat. In this case you should try not to eat at least 1 hour before you brush your teeth. Which of your snacks or meals would that involve? (Refer to Recording sheet for self-reported frequency of snacking and drinking) If you stopped that snack, what would you do instead? (Options are Substitution or Remove: Prompt for substitution of a non-erosive snack / eat snack at previous meal so feel fuller. If suggests alternative then prompt How would you remember to eat that instead? What would help you to remember? Would it mean taking something to work or school?

		If suggests “Have nothing” prompt: How easy would that be – do you think you might find yourself getting hungrier later on? What would you do then? - Prompt for not snacking again, suggest eat more at previous meal to sustain hunger.)
↓	↓	↓
Then we say what you will do instead (Note substitution or removal)	Then we say what you will do instead (Note substitution or removal)	We have found that a useful way to remember this is to make a note on this sheet. We complete it like this (Note target on prompt sheet)
↓	↓	↓
And then list all the things that will help you to make that change (Note supports already mentioned) Can you think of anything else that will help you make this change (Prompt for – tell friends and family so they don’t offer you snacks, reward self for change, put stickers on fridge / other places).	And then list all the things that will help you to make that change (Note supports already mentioned) Can you think of anything else that will help you make this change (Prompt for – tell friends and family so they don’t offer you snacks, reward self for change, put stickers on fridge / other places).	Then we say what you will do instead (Note substitution or removal)
		↓
		And then list all the things that will help you to make that change (Note supports already mentioned) Can you think of anything else that will help you make this change (Prompt for – tell friends and family so they don’t offer you snacks, reward self for change, put stickers on fridge / other places).

8.9 CORRELATIONS BETWEEN TOOTH WEAR MEASUREMENTS ANALYSED BY SURFACE

Figure 59: Correlation matrix assessing different parameters when measuring buccal surfaces of central incisors

	Volume Change	Profilometric Change	Profilometric loss	Maximum point loss
Volume Change	1	-.702**	-0.058	-.260**
Profilometric Change	-.702**	1	-0.032	.211*
Profilometric Loss	-0.058	-0.032	1	.487**
Maximum Point Loss	-.260**	.211*	.487**	1

Figure 60: Correlation matrix assessing parameters when measuring palatal surfaces of central incisors

	Volume Change	Profilometric Change	Profilometric loss	Maximum point loss
Volume Change	1	-.500**	0.012	0.176
Profilometric Change	-.500**	1	-0.138	-0.116
Profilometric Loss	0.012	-0.138	1	.629**
Maximum Point Loss	0.176	-0.116	.629**	1

Figure 61: Correlation matrix assessing parameters when measuring the occlusal surfaces of lower first molars

	Volume Change	Profilometric Change	Profilometric loss	Maximum point loss
Volume Change	1	-.725**	0.076	.306**
Profilometric Change	-.725**	1	-0.069	-0.113
Profilometric Loss	0.076	-0.069	1	.473**
Maximum Point Loss	.306**	-0.113	.473**	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

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